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Studies on the connection between physico-chemical properties and stimulating abilities of some sweet and bitter compounds

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STUDIES ON THE CONNECTION BETWEEN PHYSICOCHEMICAL PROPERTIES AND STIMULATING ABILITIES OF SOME SWEET AND BITTER COMPOUNDS

INAUGURAL DISSERTATION

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Studies on the connection between physicochemical properties and stimulating abilities of some sweet and bitter compounds

By Arne Marcström

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ABSTRACT

The effects of temperature and pH on the magnitude of the thresholds of taste in man for a number of sweet and bitter compounds has been studied. The taste quality and the stimulated area of the tongue has in each case been defined as accurately as possible. The connection between the intensity of the sensation and the strength of the stimulus has furthermore been estimated for some of the actual compounds. Considerations of the results obtained at the above mentioned experiments have led to a partition of the studied compounds in five groups. Within each group a linear relation seems to exist between the logarithms of the mean threshold values and the logarithms of the corresponding values for the solubility, represented by the number of moles present per unit of volume in saturated aqueous solutions.

I. Introduction and review of the problems

The morphological background for the discrimination of taste quality

In man the taste buds occur mainly upon the papillae circumvallatae, foliatae and fungiformes, but have been encountered, though in smaller numbers, in other parts of the buccal vacity, for example, below the tongue at the plica fimbriata (Schinkele 1942), in the soft palate (Braus 1940), upon the tonsilla palatina and

recessus tonsillotubalis (Märk 1940), on the epiglottis, and in the upper third of the oesophagus (Schinkele 1942).

According to Bielig (1939), the distribution of the taste buds upon the peripheral parts of the tongue is subject to variation, being of about the same degree within and between families. Only in certain cases, however, could similarities be noted between monozygotic twins. Thus, inheritance seems to be of no great importance in this connection.

In serial sections of the papillae circumvallatae from 38 persons (aged 20–70 years) Arey *et al.* (1935) counted the number of taste buds per "papilla and wall". The average number found was 254, yet the material exhibited great variations. The greatest number of taste buds found upon a single papilla circumvallata was 865. According to Jurisch (1922), adults possess upon their tongues, on the average, nine papillae circumvallatae with taste buds. This would imply a total of more than 2000 taste buds. Braus, on the other hand, estimated the total number of taste buds as about 2000, including 1000 on the papillae vallatae, 200 on the papillae foliatae and 800 on the papillae fungiformes.

Moncrieff (1951), presumably basing his calculations upon figures given by other authors, estimates the number of the taste buds as 9000. This shows that the estimates differ widely. As they are probably based upon restrictive material, these differences indicate considerable individual variation. This in turn can be considered as a contributary cause of the considerable differences in acuity which have often been demonstrated between observers in analyses of taste.

The appearance of the taste cells seems to be similar in different species of mammals. Detailed descriptions of the morphology of such cells have been given by Kolmer (1927), Baradi & Bourne (1953), Engström & Rytzner (1956*a*, *b*), Beidler (1961), and de Lorenzo (1958, 1963). According to Engström & Rytzner and de Lorenzo, there are in the rabbit a number of protoplasma processes, called taste villi or microvilli, which in every taste cell are situated upon the part adjoining the taste pore. These villi are supposed to be stimulated by contact with tasting substances. According to de Lorenzo (1963), the cells live only a few days and every 30 hours a new taste cell migrates from the periphery towards the centre of a taste bud. The sense cells exhibit different appearances, but this fact must not be interpreted as supporting the older hypothesis, according to which there are two differentiated types of cells, viz. sense cells and supporting cells. These differences should rather be considered as the results of the aging of the cell. According to Kolmer, among others, this view is also supported by the appearance of leucocytes within the cells, which are supposed to be compressed and pushed aside by their neighbours on aging.

The microvilli have thin cell membranes, which are stained by osmic acid (Engström & Rytzner 1956*a*), indicating the occurrence of lipids. In the cells the occurrences of an endoplasmatic reticulum, ribonucleoprotein granules, mitochondria, and Golgi apparatus have been established. The apical part of the cells also contains a great number of dense granules, which seem to correspond to the neurosecretory granules found in other tissues (de Lorenzo 1963).

An extensive investigation of the distribution of enzymes in the taste epithelium has been carried out by Baradi & Bourne (1953, 1959*a*, *b*). Bourne (1948) has established in man the occurrence of alkaline phosphatase in the walls around the taste pore and in the tissues around it. Later (1953) Baradi & Bourne established the occurrence of varying quantities of phosphatases, nucleotidases, esterase, and lipase in the taste buds and the tissues in their immediate vicinity in the rabbit.

According to de Lorenzo (1963), the nerve fibres occurring in the immediate vicinity of the taste cells of the rabbit seem to be of two different kinds. They differ from each other in their diameters and in the way in which they make contact with the taste cells. "The large fibres always seem to terminate in relationship to two or more receptor cells, i.e. they may innervate more than one receptor or may be free nerve endings, since they are between cells, near pores and more readily available to the extracellular environment." The reason for occurrence of the great number of synaptic vesicles in the nerve ends has not been discovered so far. The bud-shaped terminations of the thinner fibres are entirely enclosed by the membrane of the sense cell. They contain punctate fibrils, and small numbers of mitochondria and synaptic vesicles.

In man the distal two-thirds of the tongue with the papillae fungiformes are innervated by the chorda tympani branch of the nervus facialis, whereas the proximal third of the tongue with the papillae foliatae and papillae circumvallatae is innervated by the nervus glossopharyngeus. Fibres from taste buds in the upper parts of the oesophagus run upwards in the nervus vagus. Harris (1952) was able to follow up a large number of clinical cases in which the nervus trigeminus had been put out of action for one reason or another. In a few cases this had led to the simultaneous disappearance of the sense of taste. This may indicate that fibres from the taste buds had passed through this nerve. Thus, there may be individual differences with regard to the course of the afferent tracks. Receptors for other sensory qualities, like pain, cold, heat, pressure, and touch, which usually appear on tasting certain substances (Cohn 1914, von Skramlik 1927) are innervated by fibres from the trigeminus, glossopharyngeus and vagus (Braus 1940). Detailed descriptions of the course of the afferent tracks from the tongue to the centres of taste have been given by Börnstein (1940), Braus (1940), Hall & Blakeslee (1945), Harris (1952), Patton & Amassian (1952) and Pfaffmann (1959).

For a long time it has been assumed that the buccal cavity probably contains four functionally different types of receptors corresponding to the four qualities of taste, viz. sweet, sour, salty, and bitter. Hahn, Kuckulies & Taeger (1938) included also the alkaline taste among the primary sensations of taste (see also Henning (1927)). The following arguments seem to be in favour of the existence of the four different types:

(1) Zonal distribution upon the tongue of the four qualities of taste, viz. sweet, sour, salty, and bitter.

(2) Cocaine, applied to the tongue, eliminates one by one bitter, sweet, sour, and salty tastes. Examples of other substances producing similar effects are given by Henning (1927). According to Diamant *et al.* (1965), an extract of *Gymnema sylvestre* eliminates the neural responses in man to sweet-tasting chemicals but has no significant effect upon responses to NaCl, citric acid and quinine hydrochloride. Psychophysical experiments performed by these authors also show that the sweet taste only is eliminated.

(3) Certain fungiform papillae are sensitive to one quality of taste only (Öhrvall (1891) and Kiesov (1898), according to Henning (1927)). They were, however, found to be few in number. Recently von Békésy (1966) has obtained results which seem to indicate that single papillæ are sensitive only to one specific taste quality. Unlike Öhrvall and Kiesov, he found that only a few papillae responded to more than one taste quality.

Part of the electrophysiological examinations of single-fibre preparations in the

taste nerves of different animals throw some doubt upon the discrete nature of the taste receptors. In several papers it has been shown that the application to the tongue of substances representing different qualities of taste to man often causes an increase in the frequency of the nerve impulses (Pfaffman (1941, 1955), Cohen *et al.* (1955), Gordon *et al.* (1959), Konishi *et al.* (1961), Andersen *et al.* (1962) and others). On account of the circumstance that every fibre seems to innervate more than one taste cell (de Lorenzo 1958), these results do not permit any decision as to whether the different compounds in question stimulate the same or different receptor cells. However, in a micro-electrode study of the taste cells of the rat and the hamster, Kimura & Beidler (1961) have shown that the receptors reacted to stimuli applied to more than one quality of taste. Yet, on the other hand, fibres have also been found which on the basis of the experiments performed might most logically be called "bitter fibres" and "sour fibres" (Andersson *et al.* (1959), experiments with dogs, Cohen *et al.* (1955), experiments with cats, Iriuchijima & Zotterman (1961), experiments with dogs), "salt fibres" and "sweet fibres" (Andersson *et al.*, loc. cit., Zotterman (1959), experiments with monkeys, Iriuchijima & Zotterman loc. cit.), or even "sodium chloride fibres" (Pfaffmann (1955), experiments with rats), and "maltose fibres" (Andersen *et al.* (1962, 1963), experiments with dogs). Thus, both unspecialized and quite specialized types of receptors seem to be represented in the taste epithelium of the animal species examined. The supposition often made that the same conditions apply in man, seems, however, to be contradicted by the results obtained by von Békésy (see above).

In 1941 and 1955 Pfaffmann suggested that the discrimination of the quality of taste should be based on the cross-fibre pattern of a discharging taste nerve. This hypothesis has been discussed in some detail by Erickson (1963). When the responsiveness (impulses in the first second of activity) of each member of a sample of 13 single-fibre preparations to each of the compounds NH_4Cl, KCl and NaCl was exhibited in a diagram, it could be shown that the patterns for NH_4Cl and KCl were very similar, while that for NaCl exhibited a decidedly different appearance. Later, in behavioural experiments rats were trained, with the aid of electric shocks, to avoid drinking a solution of one of the above-mentioned salts. It then became obvious that the induced avoidance of drinking became generalized between KCl and NH_4Cl but not so much between these compounds and NaCl. These results agree well with the cross-fibre pattern theory. In this connection it may be mentioned that Beidler (1961) analysed the results he obtained in 1953 in experiments in which salt solutions of different concentrations were made to flow over the tongues of rats, while at the same time the integrated electrical responses were measured in the chorda tympani. According to Beidler, both NH_4Cl and KCl seem to act upon two, mutually independent, receptor sites, while NaCl seems to act upon one only. From the point of view of comparative physiology it is here of interest to note that many substances upon application to the tongue of man produce more than one quality of taste in succession with time or even with strength of stimulus, and that, according to Renqvist (1919), NH_4Cl and KCl can produce a bitter taste side by side with the salty.

The new hypothesis, discussed by Pfaffmann also in 1959, differs from the old one only as regards the location of the receptor sites. According to my interpretation of this new hypothesis, every receptor is a functional part of one or more of the populations of receptors which each represent one of the occurring qualities of taste, and contains types of receptor sites characteristic of the population in question. Since the number of existing types of sites on each receptor may be assumed to vary, the

individual receptors are also of different degrees of importance for the total response obtained from the different populations into which they enter. The hypothesis admits the possibility that substances creating identical sensations of taste can act on different kinds of receptor sites only on condition that these sites occur in one and the same population of receptors.

The problem of how a cross-fibre pattern can be imagined to be retained in the taste nerves in spite of the fast turnover of the cells in the sensory epithelium has been discussed by Beidler (1963) and by Davies (1963).

For a broader review of this subject, the papers of Pfaffmann (1959), Beidler (1961), Oakley & Benjamin (1966) and Zotterman (1967) should be consulted. Many interesting communications also appear in the list of more than 3000 references concerning the sense of taste, which was recently published by Pangborn & Trabue (1966).

Possible factors involved in the discrimination of taste intensity

The study of the way in which the sense of taste functions in man touches on numerous complicated problems. One of them refers to the interpretation of the information obtained from psychophysical experiments of conventional type. The processes taking place between stimulating molecules and excitable systems are transformed into a code, which is then dealt with in the taste centre.

A sensation of a certain quality stated by an observer has been imprinted upon his mind by previous experiences and, provided that Pfaffmann's cross-fibre pattern in the above representation is valid, can be taken as expressing the fact that a certain fraction of the total population of receptors has been engaged. The intensity of the sensation is in turn related to the extent to which this fraction has been engaged. Whether or not stimuli producing the same taste sensation affect the same or different kinds of receptor sites may possibly be decided by studying in each case the changes in intensity of the sensations which are produced by changes in the intensity of the stimulus. The circumstance that a certain relative increase in the intensity of different stimuli, which are experienced as being equally strong, does not in every *individual* case lead to the *same* increase in the intensity of the sensation may probably be due to the fact that the stimuli in question do not act in the same way upon the excitable system in the receptors.

In investigations carried out in order to examine the connection between physicochemical properties and the effect of taste substances upon excitable systems in receptor cells, the absolute thresholds[1] are often used as a measure of the stimulating ability. These thresholds are comparatively easily measured, and may be determined with considerable accuracy. From what has been said above, it is evident that the results have to be interpreted with caution. A comparison of the stimulating ability of the substances at arbitrary levels of the intensity of sensation may be expected to give consistent results only on condition that the substances act upon the excitable system in the same way.

It is likewise of interest to find out whether or not there is a simple or a complicated relationship between the intensity of the sensation and the extent to which the stimulus and the excitable system react upon each other. The intensity of sensation is expected to be a function of the total frequency of discharge in the taste nerves, a function which depends on the way in which the above-mentioned information

[1] The absolute threshold for a compound refers to the lowest concentration necessary to produce a sensation.

from the stimulated receptors is modified when transmitted to, and decoded in, the actual centre of sensation. Diamant *et al.* (1963) succeeded in recording the integrated response in the chorda tympani of man, when sucrose and sodium chloride solutions of different concentrations were applied to the tongue. Thus, there seem to be possibilities of determining, by means of such methods, the connection between the intensity of sensation and the frequency of the discharge in the taste nerve of man.

The total frequency of discharge is in turn a function of the intensity of the mutual reaction between the stimulus and some excitable system in the receptors. In expounding the fundamental taste equation (p. 429) Beidler (1954) supposes that there is a direct connection between the magnitude of response and the number of ions or molecules that have reacted with the receptors. As a matter of fact, the electrophysiological data obtained from the stimulation of the tongue in the rat with different salts (Beidler 1953) fit the equation. It may also be mentioned that the maximum ability of discharge of the individual nerve fibres does not constitute a limiting factor for such a direct connection. Beidler has shown that this maximum capacity is never made use of, even upon very powerful stimulation of the receptors with the above-mentioned taste solutions. In this connection it would be interesting to know how well the primary data obtained by Diamant *et al.* fit Beidler's equation. However simple the connection between intensity of sensation and strength of stimulus may be, it nevertheless furnishes no direct information about the way in which the *excitation* is achieved. With a good knowledge of the physicochemical properties of both the stimulus and the receptor, it may be possible to trace back the effects of the taste substance to basic processes.

The ability of a compound to produce taste sensations can be considered to be dependent on three factors, viz. (1) the factor of distribution, (2) its ability to influence and mode of influencing the excitable system in the receptors and (3) the possibility of its reaching the place of action without being changed in the meantime.

The last-mentioned factor depends on the tendency of the compound to react in solution in one way or the other with substances which have been introduced either before (for example dissolved gases) or after its application (components of the saliva). On the other hand, the factor of distribution is closely allied with the location of the excitable system in relation to the surrounding molecular structures and with the ability of the stimulating molecules to penetrate these structures. Each of the above-mentioned three factors can be said to be an expression of the interaction between the physicochemical properties of the substance and the affected parts of the biological system. The physicochemical properties are universally considered to be determined by the molecular structure (size, shape, charge, and participating components).

According to Danielli (1958), the cell membrane, in its simplest form, consists of a film of lipoprotein, built up in such a way that two monomolecular layers of protein enclose two "monolayers" of lipid molecules, the non-polar parts of which are orientated towards each other. This picture of the construction of the cell membrane agrees well in principle with the concept formulated by Robertson (1963) upon the basis of electron-microscope investigations. Danielli has furthermore assumed the existence of pores in the cell membrane. These pores, amongst other things, permit the passage of ions which contribute to the preservation of the potential of the cell membrane. According to Danielli (1958) and Shanes (1958), the walls of these pores are covered throughout their length by a layer of protein. Its polar groups are orientated towards the centre of the pore, while the non-polar side chains are turned towards the layer

of lipids in the middle of the membrane. The occurrence of such structures in the cell membrane may also explain why certain minor polar molecules can penetrate it at considerable speed in processes closely related to or identical with active transport. In this connection it may also be mentioned that on account of its semi-fluid condition the cell membrane seems to be adapted to the fast exchange of water. For the water molecules, however, the speed of penetration is not even approximately as great as the speed of diffusion in pure water. Thus the cell membrane constitutes an efficient barrier even to the diffusion of water molecules. According to a hypothesis of Danielli (1952), molecules which have no access to pores of suitable size penetrate the membrane by making potential energy jumps into it. In doing so, they disengage themselves from the surrounding water dipoles and add to the molecules of the membrane. From these, they are then freed again, whereupon they pass into the cell. The amount of energy required for such a transition from the water phase to the membrane phase is determined not only by the size of the molecule but also by the charge upon and the number of the polar groups present. If a great amount of kinetic energy is required, the coefficient of distribution between the two phases is small, and the speed of penetration of the substance correspondingly low. It may also be expected that the process will be dependent on the temperature, since the speed of movement of the penetrating molecules, in the direction of the membrane, can be assumed to be a function of the temperature.

In this connection it is a question of great theoretical interest whether or not dissociated or undissociated substances by their adsorption on or penetration through such a limiting surface can produce essential changes of the potential, even in low concentrations. According to Ehrensvärd (1942), the smell and taste cells may, from the purely physicochemical point of view, be considered as diphasic oil-chain systems, in which the membrane of the receptor forms the oil phase, while the plasma of the cell and the fluid occurring outside the cell constitute the two surrounding water phases. In model experiments with such systems, in which substituted benzene derivatives acted as the oil phase, Ehrensvärd demonstrated that a change in the composition of one of the water phases resulted in a change of potential which could be assumed to be localized in the actual phase boundary between oil and water. The curves relating the potential of the oil-chain system of the 1-M KCl/oil/x-M KCl type to the concentration of potassium chloride exhibit a course which is characteristic of the oil in question. The asymmetric potential was concidered as being dependent mainly upon the contribution of adsorption and distribution, but other contributions (see below) also appeared with different strengths from case to case.

On the introduction of uncharged molecules, for instance, aliphatic alcohols, at one of the surface phases, the molecules of some of these alcohols showed specific abilities to lodge between the actual molecules of the oil in question (for example salicyl aldehyde, benzaldehyde), at the same time forcing out water dipoles from this space. Since the water dipoles had decreased the electrostatic contribution of the polar groups of the oil to the dipole moment, this—and consequently also the total dipole moment of the structure of the phase boundary—was increased. This led in the actual cases to an increased negativity of the adjacent KCl solution in relation to the one at the other end of the chain. Other contributions were found to act in a similar way. The different factors which may contribute to a phase-boundary potential are according to Ehrensvärd:

Contributions to the total potential	Cause
Contribution of adsorption	Different solubilities of ions in the zone of contact
Contribution of distribution	Different solubilities of ions in the oil phase
Contribution of diffusion	Different rate of diffusion of the solubility complexes
Contribution of the dipole moment of the oil molecules	Directed orientation of the oil dipoles in the zone of contact
Contribution of induction	Induction of dipoles by the attracted ions
Contribution of neutralization	Neutralization of the oil dipoles by free water dipoles
Contribution of exchange	Exchange of oil dipoles for neutral molecules (with or without a dipole moment) dissolved in the water phase

Ehrensvärd was also able to show that the concentrations of certain substances capable of producing noticeable changes in the phase-boundary potential are of the same order of magnitude as the lowest concentrations of substances which are capable of stimulating the receptors of the sense of smell.

Apart from Ehrensvärd, several other authors have proposed more or less well-founded theories about the mechanisms behind the stimulation of the taste receptors. Beidler (1961) has given a detailed discussion and criticism of the following:

1. The enzymatic theory of Baradi & Bourne (1951), according to which the quality of the taste of a substance is determined by its ability to inhibit or to stimulate key enzymes found in the receptor cell or its surroundings. These key enzymes (one or more) are engaged in processes which generate nerve impulses in the taste fibres.

2. The adsorption theory of Renqvist (1919), according to which the intensity of the sensation is proportionate to the amount of stimulating substance adsorbed per time unit at the surface of the receptor.

3. The chemical theory of Lasareff (1922), according to which the stimulating substance reacts with a compound in the taste cell. The simultaneously formed, ionised degradation products, in their turn, cause a discharge in the afferent fibres.

4. Beidler's adsorption theory of 1954, according to which the magnitude of response is proportionate to the amount of substance adsorbed at the surface of the receptor.

The last three theories presuppose monomolecular reactions between the stimulating substance and some component of the excitable system in the receptors. But in their final interpretation Renqvist and Lasareff pay great attention to the speed at which the receptors, in psychological experiments, had been found to adapt themselves to a stimulus. According to Diamant *et al.* (1965), the shapes of the curves of adaptation obtained for sodium chloride in psychophysical and electrophysiological experiments are very similar.

Beidler has based his theory on information about the function of the sense of taste obtained by electrophysiological methods in experiments on animals. The fundamental taste equation

$$\frac{C}{R} = \frac{C}{R_m} + \frac{1}{KR_m},$$

where C represents the concentration of the taste substance, R the magnitude of the response at the concentration of C, R_m the magnitude of maximum response when all receptor sites are filled, and K is an equilibrium constant, has been derived from the law of Guldberg & Waage, and has been successfully applied to electrophysiological data.

The results of a great number of experiments carried out on man with a large number of electrolytes and organic substances show, according to Hahn (1936), that the level of the absolute threshold is influenced by the temperature in a way that is characteristic of every substance. Since, according to Hahn (1943), the dependence of the thresholds in question on the temperature can probably not be explained by the physicochemical properties of the taste substances or by the existence of a very large number of different types of receptors, the results are interpreted as depending on the effect of temperature on permeability.

The connection between the physicochemical properties and the effect of the taste substances

Cohn (1914) made the first attempt to study the connection between the chemical structure and the taste quality of a great number of substances. A similar critical review has been given by Moncrieff (1951). Cohn observed that certain substituents of the molecules, the so-called sapophoric groups, for example, $-NO_2$ and $-OH$, obviously had special abilities to cause taste sensations. Oertly & Myers (*vide* Moncrieff (1951)) assumed that sweet substances contained a "glucophore" and an "auxogluc", each of which by itself was without effect. These authors mentioned six glucophores and nine auxoglucs. Among these, no aromatic structures were listed, in spite of the fact that a great number of them were known to be capable of causing sweet taste sensations. Since certain nitro- and hydroxybenzenes have proved to be able to produce a sweet taste in man, the hydroxyls and nitro-groups may be considered as glucophores and the benzene ring as the auxogluc.

Within homologous groups of compounds an increase of the molecular weight reduces, according to Cohn, the ability of the members to produce a sweet taste. The taste may even turn into a bitter one. This he explains by the assumption that with increasing molecular weight the substances may become insoluble in water and on this account also tasteless. With the growing size of the molecule, the sapophoric groups were also thought gradually to lose their influence on the properties of the molecule. This rule is, however, subject to exceptions. Heiduschka & Komm (1925), for instance, shows that both *dl*- and *d*-alanine and sarcosine are sweeter than glycine. They say nothing about the conditions resulting from an increase in the length of the carbon chain. According to these authors, leucine which has a branched chain, has, however, a higher threshold than alanine.

Moncrieff (1951) lists the groups which Cohn and Oertly & Myers had called sapophores and glucophores, respectively. Many of the sapophoric groups contain combinations of oxygen and nitrogen atoms. On this account it may be difficult to decide which of them is of decisive importance for the taste quality of the substance. Such groups also exhibit a certain ambivalence between bitter and sweet taste, an ambivalence which is obviously also influenced by their location within the molecule.

Often a considerable strengthening of the effect of the taste substances upon the taste organs can be obtained by the introduction of side chains. Moncrieff reports that Blanksma & van der Weyden and, later Verkade, van Dijk & Meerburg

have shown that with an increase of the length of the side chain up to the propyl derivative the sweetness of 4-nitro–2-amino-phenyl alkyl ethers increases. An additional lengthening of the chain produces, according to the latter authors, a decline in the stimulating effect. In the same way Chiancone (1949) has shown that the bitter taste of esters of gallic acid is intensified with the lengthening of the carbon chain, at least from the methyl to the butyl derivative.

Certain substances containing aromatic or heterocyclic rings often have a very strong taste. Examples are the above-mentioned alkyl ethers, saccharin, dulcin, and suosan, which produce a sweet taste, and picric acid and alkaloids, which produce a bitter taste. The localization of the substituents, considered as the effective groups, seems to be of great importance for the stimulating ability of the substance.

Many authors have also stressed the importance of the stereo-structure for the taste quality of a compound and for its ability to stimulate. Heiduschka & Komm (1925) was able to demonstrate different strengths of taste for *dl*- and *d*-alanine. According to Tsuzuki & Yamazaki (1953), the α and β forms of different hexoses have different degrees of sweetness. Solms & Vuataz (1965) and according to Moncrieff, Kaneko (1939) showed that the laevo-rotatory form of certain amino acids is bitter, while the dextro-rotatory form is sweet. According to information supplied by Moncrieff, the isomers of tolylurea and *m*-nitranilines also have different tastes.

The introduction of one and the same substituent into the fundamental structure of different substances with the same taste may for some of them result in an alteration of the taste quality, while for others only the strength of the taste is influenced. Thus, Hamor (1961) showed that changes in the stereo-structure or substitution of groups in certain positions in the molecules of compounds belonging to the saccharin series often resulted in the disappearance of the present taste quality or in the appearance of a new one. While, according to Blanksma *et al.* (1946), in the series 1-chloro-, 1-bromo-, and 1-iodo–2-amino–4-nitrobenzene an increase of the sweetness of the molecule is obtained, Moncrieff reports for the series *p*-chloro to *p*-iodo saccharin a transition from sweet to bitter taste. Furthermore both 1-methoxy and 1-ethoxy nitraniline are sweet, while *p*-methoxy saccharin is sweet (Ehrensvärd 1942) and the *p*-ethoxy derivative, according to Moncrieff, is bitter.

So far it is impossible to predict the variable effects of the substituents introduced. They may be thought (1) to form a steric hindrance to or facilitate the processes of attachment or penetration, (2) to influence the density of charge and the proneness to reaction of other groups in the molecule which are suspected to be sapophores, or (3) to themselves act as sapophoric groups.

Compounds with sweet and/or bitter tastes seem to occur within a great number of groups of substances with more or less complicated stereo-configurations in aqueous solution. They range from small, strongly charged spheres, formed by an ion with its surrounding watery envelope, to the relatively complicated configuration provided with polar groups such as is found, for example, among the above-mentioned molecules with sweet or bitter taste. According to the model experiments of Ehrensvärd, the phase-boundary potential in the presence of small charged particles is formed mainly by the contributions of adsorption and distribution, while the contributions of dipole moment and of neutralisation gain in importance in the presence of more complicated molecules. Provided that similar conditions exist at the interfacial region constituted by the receptor membrane, it may be most promising to start the study of the connection between structure and effect by making comparisons between the

stimulating ability of the substances in one of the above-mentioned groups, i.e. in the groups of electrolytes or substances with a more or less complicated three-dimensional structure.

Plan of investigation

For the present paper the author decided to make comparisons between the stimulating abilities in human observers of all the obtainable benzene derivatives containing nitro substituents, hydroxy substituents and nitro and hydroxy substituents in combination and of the smaller alkyls ($C_1 - C_3$) containing one of the substituents mentioned. Benzene has been chosen as a basic structure, since its stereo-configuration is known in detail and is not noticeably altered by the introduction of the substituents. It is then possible to examine the effect of the positions of the substituents in the molecule upon the action in the sense organs.

The absolute thresholds for some of the substances in question have admittedly been already determined by other authors. The results obtained at different laboratories are, however, not immediately comparable. This is due to the use of different psychophysical methods and also to the variation between different observers with regard to the number of receptors constituting the actual populations of receptors. For this reason also the acuity of the observers can be expected to vary considerably (see p. 423). Also the information about the experimental conditions, for example, the temperature and the pH in the buccal cavity, is often incomplete or entirely missing.

The importance of the above-mentioned conditions for the level of the absolute thresholds of the aromatic derivatives has also been studied. In cases in which metabolic processes are involved or the penetration of the substance in question through the cell membrane is dependent on potential energy jumps (cf. Danielli 1952), an effect of the temperature may be expected. An effect of pH may also appear if the stimulating molecules and/or the excitable system are subject to proton exchange in aqueous solutions.

The observers have, of course, defined the taste quality of the substances as accurately as possible. For the study of the connection between the structure and the effect of bitter and/or sweet substances, such information is decisive. But even if the taste quality is the same, it is not out of the question, as was mentioned earlier (page 426), that the substances may influence the excitable system in different ways. For this reason comparisons between the stimulating ability at different levels of the intensity of the sensation have been carried out, though only for a restricted number of aromatic derivatives. The relationship between stimulating potency and number of moles per unit of volume in saturated aqueous solutions of the compounds tested has also been examined.

II. Methods

A. *Introduction*

It has already been mentioned that there are probably great variations between different individuals with regard to the number of taste buds in the oral cavity, and thus also with regard to their sensitivity to taste stimuli. The level of the absolute thresholds reflecting this sensitivity is furthermore subject to variations resulting from several factors which can be classified under the headings "method applied" and "experimental conditions". Such factors are, for instance:

1. The mode of application of the stimulus to the sensory surfaces.
2. The stimulated area of the tongue and its state of rest or movement.
3. The amount of solution applied.
4. The duration of application.
5. The possibilities of removing a preceding stimulus.
6. The size of the steps in the series of concentrations presented to the observer, especially that covering the threshold value.
7. The physicochemical state of the substance investigated under the pH conditions prevailing at the excited sensory surface.
8. The temperature of the excited sensory surface.
9. The composition of the solution with respect to ions and molecules other than those of the investigated substance, when excitation arises.
10. The degree of training attained by the subject with the method employed.
11. The physical and psychical condition of the subject at the time of the experiment.
12. The kind of food and the time since its ingestion by the subject.
13. The time that has elapsed since smoking or the use of pharmaceutical preparations which may influence the sensitivity of the subject.
14. The appearance of sensations other than that studied, for example, smell, warmth, cold, touch, etc., which may alter the subject's ability to discriminate between the situations "no taste of the solution observed" and "taste observed".

The importance of some of the above factors for the level of the thresholds has been pointed out by several authors, for example, Gibson & Hartmann (1919), Sale and Skinner (1922), Henning (1927), Richter & McLean (1939), and others. I do not intend to go into a detailed discussion of the numerous methods employed for the determination of absolute threshold values. As a rule, many circumstances which on account of our incomplete knowledge of the function of the taste apparatus may be considered as important for the results are not stated. For this reason, I have selected, by experiment, the method described below, which is quite rapid, and at the same time makes possible a rough estimation of the conditions prevailing during the experiments. Besides the theory underlying this classical method of measuring the threshold, other theories, such as the signal-detection theory and the neural-quantum theory have recently appeared in the literature. In experiments aiming at the estimation of the relative stimulating ability of compounds under different experimental conditions, it seems reasonable to assume that the response criteria of the observers is fairly constant. Therefore it also seems to be of little account which type of threshold is selected.

B. *Experimental procedure*

In the taste experiments described in this paper, for each determination of the threshold every single test subject (hereinafter called "observer") was asked to express an opinion about seven liquid preparations contained in dark-brown, almost opaque test-tubes. Of these seven preparations, six contained aqueous solutions of the substance the threshold value of which was to be determined, while the seventh contained only water. The concentrations of the solutions were arranged in a geometrical series, the highest and lowest values having been chosen so as to straddle the absolute threshold of the observer. For each determination of thresholds

the seven test-tubes were presented to the observer in random order, the order being arranged by the conductor of the experiments or his assistant.

Prior to the assessment of each liquid preparation, the observer was required to rinse his mouth with water four times in succession. After the assessment of the seventh sample the experiment was terminated by four rinsings.

All the solutions and the water required for rinsing the mouth were presented to the observer in a test-tube rack (here called "the rack"). In the rack the items were arranged in eight rows. Rows 1–7 each held four tubes of clear glass, each containing 15 ml of water, and a brown test-tube containing 15 ml of a liquid preparation. The eighth row contained four clear-glass tubes with 15 ml of water in each of them.

The observer was told to taste the contents of the test-tubes in the order in which they had been arranged. Prior to the experiment, he was told that there was also water among the liquid preparations which he was required to judge, but he was not informed about the number of tubes containing water.

In order to make the experimental conditions for determining the threshold values of the different substances as uniform as possible, all observers were allowed the same length of time for tasting the contents of each test-tube. During the experiment a buzzer controlled by an electric clock produced a low sound every 6 seconds. At one of these signals the observer drained the contents of the first tube in row 1, and by forceful movements of the tongue and cheeks caused it to circulate around the mouth. In this way the rinsing water came quickly into intimate contact with the taste epithelium of the oral cavity. At the next signal the mixture of saliva and water was discharged into a pail placed near the rack. This procedure was repeated in unison with the acoustic signals, using the remaining 38 test-tubes in the rack. In this way the observer had four opportunities of becoming acquainted with the taste which the water might have or with other irrelevant sensations which accompanied the experiment. Only then did he taste the preparation in the first brown test-tube. After this he spat out the mixture of saliva and preparation and wrote a statement in the 6 seconds available before he was to carry out the first of the four rinsings with water. The statement took the form of writing down the figure 1, if the liquid preparation had caused a taste sensation differing from that which might have been created by water, and the figure 0, if this was not the case. The observers were told to give an objective and independent opinion about every single preparation. No alteration of a statement was permitted later on. The observer was also told to note down, if there was an opportunity, the first letter of the word describing the taste sensation produced by the liquid preparation he had just tasted. When all the liquid preparations in one rack or in a set of racks had been tasted, the observer was required to mark upon an outline sketch of the tongue the position of the stimulated receptor surface. In this connection he was also given the opportunity of making a more detailed statement about his taste sensation and of putting down on paper other observations which might be of interest to the conductor of the experiments.

Some of the tested substances proved to have a strong smell. With the aim of eliminating as much as possible the possible influence of this factor upon the results of the experiments, the observers were required to wear a nose clamp during all taste experiments. This clamp effectively prevented the inward or outward passage of air through the nostrils. The observers breathed through their mouths in the intervals between the tastings. They were asked to report cases in which sensations of smell were observed. In the course of the experiments described in this paper no sensations of smell were reported.

1. *Procedure and experimental conditions in the testing of observers in group I*

Group I consisted of Mrs. Britt Öhman-Saks, a laboratory assistent, and the author. Both could be considered as being trained, since they had acted as observers in different taste experiments almost 6 days a week for about 4 months preceding the present experiments.

Altogether 26 substances were tested. They are listed in Table 1, col. 1. The order (col. 4) in which the experimental series of the different substances were to be tasted during the actual test period was decided with the aid of a table of random numbers. In the course of determining the thresholds for a compound, the observers were offered—in the majority of cases several times—racks containing tubes with (*a*) distilled water at room temperature, (*b*) distilled water at about 37°C, (*c*) tap-water at room temperature, and (*d*) tap-water at about 37°C, for rinsing and as solvent in the actual solutions which were to be judged. The number of series of concentrations assessed by each observer under each of these experimental conditions is given in cols. 5–8.

During the experiments the two observers alternately acted as conductors of experiments to each other. Each of them decided on the day preceding the experiment (1) the randomized order of the racks and (2) the randomized order of concentrations of the relevant substance on each rack in which they were to be presented to the other observer.

Immediately before beginning the taste experiments, the two observers prepared the first two racks for each other. The racks with samples intended to have a temperature of about 37°C at the time of tasting were placed in a thermostat-controlled water-bath within reach of the observer.

The observers simultaneously started the tasting at least one hour and usually one and a half hours after the last meal.

The tasting of the contents of the first rack was followed by a respite of about 3 minutes before the next rack was served. During this time the observers arranged the third rack for each other. Rinsing water and solutions were tapped from storage bottles. Then the racks were left for temperature equilibration for about 11 minutes. The test-tubes, when filled to the brim, contained 15 ml. Their filling was therefore quickly accomplished.

Since the observers never knew in what order the preparations of liquids were arranged, it was easier to avoid errors due to habit or expectation. Before the beginning of the experiments the pH of the solutions made up with distilled water and of the distilled water used for rinsing was adjusted to about 5.1 (radiometer type PHM 22r, measuring accuracy between 0.05 and 0.02 pH, probably in the vicinity of 0.02). This was done by adding dropwise suitable amounts of 1 *M* HCl or NaOH. In the course of the day the change in the concentration of hydrogen ions was inconsiderable, since solutions and rinsing water were stored in well-stoppered bottles and came into contact with the surrounding air only when new concentration series were prepared.

The tap-water that was used as solvent and for rinsing during the taste experiments was tapped on the preceding evening into storage bottles holding 50 litres and was allowed to attain room temperature in the course of the night. Each morning, when experiments were carried out, the pH was determined. The values for each series of experiments are given in Table 1, col. 9. It should be observed that experiments Nos. 22, 24, 25 and 26 (cf. col. 4) were performed with tap-water, the pH of

Table 1. Names and qualities of compounds tested and number of experiments performed

The meaning of the superscripts

	1	2	3

	Compound			
No.	Name used	Synonym	Producer	Quality
1	Benzene		Stockholms gasverk	Pro analysi
2	Nitrobenzene		Stockholms gasverk	Pro analysi
3	1.2-Dinitrobenzene		Schuchardt	Puriss.
4	1.3-Dinitrobenzene		Fluka	Purum
5	1.4-Dinitrobenzene		Eastman	Pro analysi
6	1.3.5-Trinitrobenzene		Eastman	Pro analysi
7	Phenol		Merck	Pro analysi
8	Pyrocatechol	1.2-Dihydroxybenzene	Merck	Pro analysi
9	Resorcinol	1.3-Dihydroxybenzene	Merck	Pro analysi
10	Hydroquinone	1.4-Dihydrobenzene	Schuchardt	Puriss
11	Pyrogallol	1.2.3-Trihydroxybenzene	Baker	Pro analysi
12	Phloroglucinol	1.3.5-Trihydroxybenzene	Merck	Pro analysi
13	*o*-Nitrophenol	2-Nitrophenol	Merck	Pro analysi
14	*m*-Nitrophenol	3-Nitrophenol	Merck	Pro analysi
15	*p*-Nitrophenol	4-Nitrophenol	Merck	Pro analysi
16	2.4-Dinitrophenol	α-Dinitrophenol	Merck	Pro analysi
17	2.5-Dinitrophenol	γ-Dinitrophenol	Merck	Pro analysi
18	2.6-Dinitrophenol	β-Dinitrophenol	Merck	Pro analysi
19	2.4.6-Trinitrophenol	Picric acid	Merck	Pro analysi
20	4-Nitroresorcinol		Chem. Inst., Uppsala	Melting p. 118–121
21	Benzaldehyde		Merck	Puriss.
22	Vanillin		British Drug Houses	Pro analysi
23	Veratrole		Merck	Puriss.
24	Quinine hydrochloride		British Drug Houses	Puriss.
25	Phenylthiourea	Phenylthiocarbamide (P.T.C.)	British Drug Houses	Pro analysi
26	Sucrose	Sacharose	Mallinckrodt	Pro analysi
27	Sodium chloride		Merck	Pro analysi
28	Sodium nitrite		Riedel de Haën AG	Pro analysi
29	Potassium nitrite		Merck	Pro analysi
30	Nitromethane		Eastman	Purum
31	Nitroethane		Eastman	Purum
32	1-Nitropropane		Eastman	Purum
33	2-Nitropropane		Eastman	Purum
34	Methanol	Methyl alcohol	Merck	Pro analysi
35	Ethanol	Ethyl alcohol	Systembolaget, Stockholm	99.5 %
36	2-Propanol	Isopropanol	Merck	Pro analysi

by each observer in groups I and II under the given different experimental conditions. in col. 10 is explained in the text.

4	5	6	7	8	9	10	11	12	13	14
	Group I (Mean room temperature 21.7°C; Mean humidity 40.7 %) Number of racks tasted by each observer					*Group II* (Mean room temperature 22,4°C; Mean humidity 33.2 %) Number of racks tasted by each observer				
	Distilled water (pH 5.1)		Tap-water				Distilled water			
Exptl. series No.	Temp. 20.4°C	Temp. 36.9°C	Temp. 20.4°C	Temp. 36.9°C	pH	Exptl. series No.	pH 5.1, Temp. 20.8°C	Exptl. series no.	pH 7.80, Temp. 20.8°C	Wavelength (nm) at which conc. of tested solutions was spectrophotometrically checked
5	1	1	1	1	7.50	A 21	See text			254
22	5	5	5	5	7.90	A^1 11	2	C 13	2	267
25	5	5	5	5	7.95	A^1 13	2	C 16	2	266
7	5	5	5	5	7.35	A 3	2	C 14	2	240
26	5	5	5	5	8.00	A^3 6	2	C 15	2	266
16	5	5	5	5	7.40	A^1 10	2	C 10	2	228
10	5	5	5	5	7.50	A^1 14	2	C 8	2	270
20	1	1	1	1	7.50	A 21	See text			226.5
14	5	5	5	5	7.35	A^3 1	2	C 3	2	273
						Replicate	2			
						Replicate	2			
						Replicate[2]	2			
						Replicate	2			
						Replicate	2			
6	5	5	5	5	7.50	A^2 8	2	C 5	2	269
19	5	5	5	5	7.35	A^3 5	2	C 4	2	227.5
17	5	5	5	5	7.40	A 17	2	C 12	2	266
3	5	5	5	5	7.35	A^1 7	2	C 11	2	259, 370
21	5	5	5	5	7.50	A^1 9	2	C 6	2	289
12	5	5	5	5	7.40	A 12	2	C 7	2	266, 345
4	5	5	5	5	7.40	A 20	2	C 9	2	360
15	1	–	5	5	7.50	A 19	2	C 2	2	365
18	1	–	5	5	7.50	A 21	See text			375
9	5	5	5	5	7.40	A^1 4	2	C 1	2	353
11	–	5	–	5	7.30					270
1	1	1	1	1	7.45	A 21	See text			249
23	1	–	–	–		A 21	See text			316
8	1	1	1	1	7.40	A 21	See text			272
13	5	5	5	5	7.30	A 15	2			242
24	5	5	5	5	7.90	A^2 2	2			247
2	5	5	5	5	7.40	A 16	2			
						A 18	2			
						B 2	2			
						B 3	2			
						B 9	2			
						B 7	2			
						B 6	2			
						B 1	2			
						B 5	2			
						B 4	2			
						B 8	2			

which was considerably higher than in the other experiments. The Institute of Zoophysiology in Uppsala draws its tap-water from a water tower which on these occasions had evidently obtained an ample supply of water from some pumping plant supplying particularly alkaline ground water. The mean value of the temperatures of the liquids in the first and the last rack was assumed to represent the average temperature for the day for all samples assessed under similar conditions. The mean value of the average temperatures for the samples kept at room temperature (see heading in Table 1, cols. 5 and 7) and in the water-bath (cols. 6 and 8), on the days when such samples were used for the determination of threshold values, have been calculated as 20.4°C and 36.9°C respectively (the number of determinations, n, was 25 in each case). The corresponding standard deviations were 0.5° and 0.1°C respectively. As can be seen, there is only an inconsiderable variation of temperature in the samples of liquid in the racks which had been placed in the water-bath. The variation is greater among the series kept at room temperature, resulting from fluctuations of this temperature during the same time (mean value 21.7°C, standard deviation 0.7°C).

The humidity of the air in the laboratory was measured every day by means of a hair hygrometer. The arithmetical mean and the standard deviation for the test period were calculated as 40.7 and 3.6 % ($n = 26$). The comparatively high humidity can be explained by the evaporation from the relatively large surface (0.5 m²) of the thermostat-controlled water-bath.

Finally, it may be mentioned that on the days on which the taste experiments were performed, no symptoms of any illness which could be thought to influence the results of the experiments were noticed in the observers. Mrs. Britt Öhman-Saks did not smoke during the test period in question and may be described as a non-smoker. The author smoked very moderately and never before the termination of the experiments for the day.

2. *Procedure and experimental conditions in the testing of observers in group II*

The observers in group II were 18 students working for their pass degrees in zoology at the University of Uppsala. All of them were without previous experience of taste experiments. These 18 students were joined by two test subjects (Mrs. Öhman-Saks and the author), who at this time were already trained tasters.

All the tested substances are shown in Table 1 (p. 436), col. 1. In all experiments distilled water was used both as solvent and for rinsing. Before the composition of each rack the pH of solutions and rinsing water was adjusted to 5.1 or 7.8 by the addition of suitable amounts of HCl or NaOH. Cols. 11 and 13 give the number of the racks assessed on each occasion of the testing of an observer and compound respectively.

According to the assessments by the persons in group I, the substances benzene, pyrocatechol, 2.6-dinitrophenol, benzaldehyde, veratrole and vanillin proved to be unable to create taste sensations. The observers in group II were asked to give a more detailed description of the sensations created by the substances in question in a concentration determined beforehand. A solution of each of the substances was introduced, together with the check sample, into a rack in the way described on p. 434. When judging each of them, the observers wrote down the first letter of the word designating the sensation or sensations which the preparation might have caused. On the occasion in question two racks were tasted one after the other.

The sequence in which the other substances were tested during the periods March-April (A), May (B), and April-May (C) is given in cols. 10 and 12.

In connection with the first randomization it was decided that threshold determinations for the first tested substance, viz. resorcinol, should be repeated at equal intervals (every 10th experiment performed) as long as the taste experiments lasted. These experiments are designated as replicates in col. 10. Thus the threshold for resorcinol was determined on altogether six occasions. Resorcinol has therefor not been included in the randomization of compounds tested in periods B and C, except once at a new pH (7.8).

Every observer had at his disposal a test-tube rack with a complete set of test-tubes for rinsing the mouth. The tubes were thus used twice in succession. The liquids to be assessed were poured into brown test-tubes, which were used once only on each occasion of testing.

These experiments were conducted by Mrs. Öhman-Saks and the author. Immediately before the beginning of the taste experiments, these two prepared the first rack for each of the observers. In addition to this, tubes with liquids belonging to the second rack to be tested were placed in an otherwise empty test-tube rack in front of the seat of the observer in question. The tubes had been arranged at random and placed so that they could be transferred without mistake to the test-tube rack with rinsing water, once the first rack had been assessed. The conductors of the experiment did not know the sequence of the liquids in the racks which they themselves were to taste.

The observers started simultaneously, by assessing the first rack in unison with the acoustic signals. This assessment was followed by a respite of about 4 minutes. During this time each observer himself refilled the tubes of rinsing water in his own rack with distilled water from storage bottles, and replaced tubes the contents of which had already been assessed with tubes made ready for rack No. 2. As all tubes had a capacity of *c.* 15 ml, their filling did not take much time.

After the termination of the experiments every observer had to enter on a duplicated form (1) his state of health, (2) the time that had elapsed since smoking, (3) the time since the last meal, (4) the position of the particularly stimulated tongue area, and (5) a more detailed description of the taste quality of the substance, as well as other observations made during the experiment.

For each occasion of testing, each observer received a renumeration of 1 Swedish crown. Occasionally it happened that one or two of the observers were prevented from attending the ordinary testings listed in Table 1 col. 10 on account of temporary illness or for other reasons. Such situations arose altogether 22 times and have been marked by a superscript next to the number of the experimental series in question. This figure indicates the number of absentees on the particular occasion. When, after recovery, the observers presented themselves again for the taste experiments, they were given freshly prepared concentration series of the substance which they were due to taste, according to the previously established scheme. In these experiments, conditions such as humidity, temperature of the room, temperature of the assessed solutions, their concentrations and their pHs were not exactly identical with those which obtained when the other persons in the group were tested. But even before the presentation of the series the conductor of the experiments was able to adjust the three last-mentioned factors to such an extent that they agreed very closely with those which obtained when the main body of the group had been tested. For this reason, the estimations of the experimental conditions given below are based on data collected during the ordinary testing of each substance.

Among the observers only six were smokers. These were asked to refrain from smoking for 30 minutes before every test.

The time which elapsed between the last meal and the beginning of the testing was usually 1½ hours, and was never less than 1 hour.

The temperature of the assessed solutions, the temperature of the room, and the humidity were determined in principle in the way already described in the preceding chapter (p. 438), but the measurements were carried out only once, prior to each testing. These data have been used as variables in the calculation of statistics representing the entire test period (cf. Table 1, cols. 11 and 13). The arithmetical mean and standard deviation for the temperatures of the solutions were 20.8°C and 0.7°C, for the temperature of the room 20.4°C and 1.0°C, and for the humidity 33.2 and 2.0 %, n being 51 in all the cases. In the further discussion of these experimental conditions, reference will be made to these data.

C. *Preparation of solutions*

Each of the solutions used in testing was prepared by dilution of a stock solution of suitable concentration. For the preparation of the latter the required quantity of the substance was weighed on a Mettler balance. On each occasion a new package of the substance in question was opened. The quality of unstable chemical compounds, for example, the di-hydroxy benzenes, may suffer from prolonged storage in opened packages.

After the termination of the day's taste experiment, we usually checked that the tasted solutions had contained the intended quantity of substance by means of a Beckman spectrophotometer, model DU, using a suitable wavelength (see below) within the range 210–635 nm. The concentration, calculated on the basis of weighing, was considered representative of the tasted solutions used, except in cases in which the spectrophotometric measurements revealed major deviations. A deviation of 5 % from the expected concentration was considered admissible, since it was, as a rule, necessary to dilute the solutions prior to measuring of the extinction. In only one single case was it found that the conductors of the experiments had made incorrect dilutions in preparing the stock solution.

D. *The stability of the tested substances in aqueous solutions of different pH*

Before the start of the taste experiments the absorption spectra of aqueous solutions (distilled water and tap-water, respectively) of the substances Nos. 1–25 listed in col. 1 of Table 1 had already been measured within the above-mentioned range of wavelengths by means of a Beckman spectrophotometer, model DK 2. Repeated determinations at different times were carried out on solutions of different ages, pHs, and concentrations.

The results showed that pyrocatechol, hydroquinone, pyrogallol and phloroglucinol are unstable within certain pH ranges in dilute solutions contained in open vessels.

In concentrated solutions the spectrum of pyrocatechol changes very slowly, presumably because the oxidation of the substance ceases after the consumption of the oxygen present in the solution. Thus solutions of pyrocatechol can be kept for several days in well-sealed containers without any noticeable change of the absorption spectrum.

Also at a pH below 7.5 the absorption spectrum changes very slowly (almost unnoticeably after 2 hours) in dilute solutions and open vessels. This is probably because the substance is present mainly in its undissociated form ($pK_a = 9.85^{20°}$), so that its reducing ability is diminished to a corresponding degree.

With regard to hydroquinone, the conditions are similar, but the changes of the absorption spectrum take place at a much faster rate. This is due to the fact that the compound is a powerful reducing agent, and easily changes into quinone. Stock solutions can be kept in closed vessels for at least 12 hours without the appearance of noticeable changes in the spectrum. As soon as the solution is diluted, a decrease in the absorption takes place with time. At the peak at 288 nm the extinction drops *c.* 8 % in 15 minutes (pH 8.28). At a lower pH this change is, however, much slower, viz. a drop of *c.* 5 % in 15 minutes at pH 6.58. At the wavelength of 269 nm the extinction was of about the same magnitude all the time during the measuring. A check on the original content of the solutions was carried out at this point.

The changes in the absorption spectrum of phloroglucinol with time are possibly connected with the oxidation of the compound or a change into more or less complex compounds of unknown composition. Their contribution to the total extinction can, of course, not be estimated. At a pH below 6.45 no noticeable changes take place in dilute solutions, at least in the course of the first 48 hours. Even at pH 6.9, however, the extinction rises at the maximum at 266 nm by 60 % in 2 hours or *c.* 7.5 % in 15 minutes. On account of the fact that the absorption greatly increases with time within the entire range of wavelengths examined, the original concentration of the solutions cannot be determined by means of the spectrophotometer.

The absorption spectrum of pyrogallol proved stable for several hours in dilute solutions with pH below 5.5. Even at pH 6.1, however, the extinction rose at the maximum at 266 nm by *c.* 3 % every 20 minutes, and at pH 7.88 by *c.* 100 % every 20 minutes. The absorption spectra for solutions of the same concentration but of different ages intersected at 227.5 nm, where the original concentration of a solution of unknown strength could thus be checked.

The extinctions of solutions of the other substances tested have been measured at a suitable maximum or at a wavelength at which the absorbancy index is independent of the hydrogen-ion activity (Table 1, col. 14). Lambert-Beer's law is valid within the ranges of concentration examined for all wavelengths at which the magnitude of the extinction proved to be independent of the age of the solutions.

When the thresholds for the above mentioned, unstable substances, hydroquinone, pyrogallol, and phloroglucinol were determined in group II, the solutions were presented as quickly as possible after dilution. The age of a diluted solution at the start of the taste experiments is estimated as having been 10–15 minutes. On the occasion of the testing of group I, however, about $3\frac{1}{2}$ hours elapsed from the time of the preparation of the solutions to the time when the last liquid preparations were tasted. With a view to reducing the speed of the oxidizing processes and keeping the pH constant, the solutions were kept in closed vessels, as has already been mentioned (p. 435). The changes in the level of the absolute thresholds in the course of the testings are discussed against the background of the observations made with regard to the proneness to reaction of the substances (pp. 471–472).

III. Calculation of mean threshold values

Observers in group I

Table 2A and B contains the judgements of the observers on each presented series of the first compound examined which gave rise to a taste sensation (sucrose) and which will form our type example.

Col. 1 contains the concentrations of the samples assessed in each series. The observers were offered geometrical series of concentrations, each concentration being *c.* 20 % stronger than the one immediately preceding. When only the temperatures of the solutions differed, identical concentration series could be used.

In the headings of cols. 3, 5, 7 and 9*a–e* will be found the order in which the different concentration series were tested. The judgement of the observers has been entered for each concentration and series. The figure 1 indicates that on the particular occasion the concentration in the corresponding line of col. 1 had a taste that differed from that of the rinsing water. The figure 0 indicates that this was not the case (cf. p. 434). Superscripts in connection with the 1's and 0's in cols. 3, 5, 7, and 9 indicate the order in which the corresponding liquid preparations had been tasted.

I have defined the absolute threshold as the arithmetical mean of the concentration assessed as 0 and the first of the following higher concentrations which has been assessed as 1. The class mean for each concentration step in the assessed concentration series containing sucrose has been given in col. 2. In the following description I shall show how this definition was applied in practice.

As is clear from p. 434, the observers were not allowed to guess in the assessments. The assessments made may be wrong (misleading) for the following reasons:

(1) In giving his opinion the observer may have interpreted irrelevant sensory perceptions as taste sensations and *vice versa*. (2) A mistake may have been made in the entry of an observation. (3) The observer may by mistake have been given another concentration than that intended.

We can form an opinion of the frequency of erroneous assessments by studying the number of cases in which the control test with water was marked with the figure 1. Such haphazard errors in assessment proved to occur in *c.* 3 % of the cases.

Table 2 also shows that, as a rule, the strongest concentrations in a series were allotted the figure 1, while weaker concentrations and the control test were marked with 0. In a small number of cases, however, it happened that among three neighbouring, fairly weak concentrations the observer assigned a 0 to the two stronger, while he noted a 1 for the weakest (Table 2A and B, col. 3*d*). The frequency of the series assessed in this way is *c.* 2 % of the total number, i.e. roughly the same as the frequency of erroneous assessments of the control test. On this account it appears probable that the last-named concentration was erroneously assessed. For this reason, no account has been taken of such an assessment in calculating the level of the threshold.

It also happened that of two neighbouring, fairly weak concentrations the stronger was assessed as 0, while the weaker was given a 1 (see, for example, Table 2A, col. 7*e*, and Table 2B, cols. 3*b*, 5*d*, and 7*c*). Here either or both assessments may be misleading, but both may also be correct. Since *c.* 15 % of the total number of series was assessed in this way, it appears probable that, apart from the factor responsible for the misinterpretation, some other factor was at work. It is, for instance, conceivable that on the occasion in question the variation of the absolute threshold

Table 3. Assessments of series of concentrations of sucrose made by observer No. 1 (Table 2A) and observer No. 2 (Table 2B).

The class mean represents the arithmetical mean of two neighbouring concentrations tasted. The presence or absence of taste in a solution is marked by the figures 1 or 0 respectively. The meaning of the superscripts in cols. 3, 5, 7 and 9 is explained in the text on p. 442.

1	2	3 a	3 b	3 c	3 d	3 e	4	5 a	5 b	5 c	5 d	5 e	6	7 a	7 b	7 c	7 d	7 e	8	9 a	9 b	9 c	9 d	9 e	10
		Solvent "distilled" water, pH 5.1												Solvent tap-water											
		Temp. 20.4°C						Temp. 36.9°C						Temp. 20.4°C						Temp. 36.9°C					
Conc. of solutions tasted, $\times 10^3$ *M*	Class mean, $\times 10^3$ *M*	Assessments of concentrations in series tasted as *Nos.* 2, 9, 11, 13, 17					Thresholds per class mean	Assessments of concentrations in series tasted as *Nos.* 4, 6, 7, 8, 16					Thresholds per class mean	Assessments of concentrations in series tasted as *Nos.* 3, 5, 14, 18, 20					Thresholds per class mean	Assessments of concentrations in series tasted as *Nos.* 1, 10, 12, 15, 19					Thresholds per class mean
A. *Observer No. 1*																									
14.4		1^1	1^3	1^2	1^2	1^1		1^1	1^3	1^7	1^6	1^5		1^4	1^1	1^5	1^2	1^4		1^7	1^3	1^2	1^5	1^2	
	13.2																								
12.0		1^7	1^4	1^5	1^4	1^4		1^4	1^7	1^2	1^4	1^3		1^2	1^5	1^4	1^4	1^1		1^6	1^7	1^4	1^3	1^6	
	11.0																		1						
10.0		1^2	1^1	1^6	1^7	1^5		1^7	1^5	1^4	1^1	1^2		1^7	1^3	1^3	0^7	0^7		1^2	1^2	1^7	1^4	1^5	
	9.17						3						1						2						1
8.33		0^5	1^6	0^7	0^3	1^7		0^3	1^2	1^3	1^7	1^1		0^3	1^4	0^1	0^3	1^6		1^4	1^5	1^5	0^1	1^4	
	7.64						1						4						1						2
6.94		0^6	1^7	0^4	0^6	0^6		0^6	0^4	0^5	0^5	0^6		0^5	1^6	0^7	0^6	0^3		1^1	0^1	1^6	0^2	0^3	
	6.37						1												1						2
5.79		0^4	0^5	0^3	1^1	0^3		0^2	0^1	0^1	0^2	0^7		0^1	0^7	0^6	0^5	0^2		0^5	0^4	0^1	0^7	0^7	
0 (control)		0^3	0^2	0^1	0^5	0^2		0^5	0^6	0^6	0^3	0^4		0^6	0^2	0^2	0^1	0^5		0^3	0^6	0^3	1^6	0^1	
		Nos. 1, 3, 5, 7, 10						*Nos.* 2, 9, 13, 16, 20						*Nos.* 4, 6, 8, 18, 19						*Nos.* 11, 12, 14, 15, 17					
B. *Observer No. 2*																									
17.3		1^2	1^1	1^3	1^4	1^5		1^7	1^4	1^2	1^6	1^7		1^5	1^4	1^5	1^7	1^5		1^7	1^1	1^1	1^6	1^4	
	16.4																								
14.4		1^5	1^3	1^5	1^1	1^4		1^4	1^6	1^1	1^1	1^4		1^2	1^3	1^1	1^3	1^4		1^5	1^4	1^6	1^4	1^2	
	13.2						3						2						1						2
12.0		1^6	0^2	1^6	0^6	0^2		1^6	1^7	1^7	0^7	1^3		1^4	1^6	0^4	1^5	1^7		1^1	1^7	1^5	1^1	1^7	
	11.0						2												3						2
10.0		0^1	1^6	0^7	0^2	0^3		0^1	1^3	0^4	1^5	1^2		0^7	0^7	1^7	0^2	1^1		1^2	0^5	1^7	0^3	1^6	
	9.17												3						1						2
8.33		0^7	0^5	0^2	1^3	0^6		0^3	0^5	0^5	0^2	0^1		0^6	0^5	0^6	0^4	0^6		1^4	0^3	0^4	0^7	0^1	
	7.64																								1
6.94		0^4	0^7	0^4	0^5	0^1		0^5	0^1	0^3	0^4	0^5		0^1	0^2	0^3	0^6	0^3		0^6	0^2	0^2	0^5	0^5	
0 (control)		0^3	0^4	0^1	0^7	0^7		0^2	0^2	1^6	0^3	0^6		0^3	0^1	0^2	0^1	0^2		0^3	0^6	0^3	0^2	0^3	

Table 3. Statistics calculated and results of analyses of variance made on threshold experimental

"Distilled" and tap-water are the solvents used in the solutions tasted and the temperature is that of deviation, C.V. = coefficient of variation expressed as a percentage, n = number of variates (absolute The symbols ***, **, * and no asterisk in the

1	2	3	4	5	6	7	8	9	10

		"Distilled" water							
		Temp. 27°C				Temp. 35°C			
Compound tested	Observer No.	n	$\bar{X} \times 10^6 M$	$s \times 10^6 M$	C.V.	n	$\bar{X} \times 10^6 M$	$s \times 10^6 M$	C.V.
Nitrobenzene	1	5	174	42.8	24.6	5	166	30.0	18.0
	2	5	168	41.0	24.4	5	175	45.5	26.0
	1+2								
1.2-Dinitrobenzene	1	5	72.2	14.6	20.3	5	76.9	9.93	12.9
	2	5	77.4	14.0	18.1	5	79.5	6.84	8.61
	1+2								
1.3-Dinitrobenzene	1	5	61.8	5.92	9.56	5	76.1	7.12	9.35
	2	5	68.7	8.44	12.3	5	59.9	8.58	14.3
	1+2								
1.4-Dinitrobenzene	1	5	62.3	11.5	18.5	5	51.2	3.94	7.68
	2	5	51.2	3.94	7.68	5	49.5	4.82	9.74
	1+2								
1.3.5-Trinitrobenzene	1	5	14.2	1.42	10.0	5	13.8	2.03	14.7
	2	5	12.0	2.54	21.1	5	13.4	2.40	18.0
	1+2								
Phenol	1	5	3,210	475	14.8	5	2,970	228	7.68
	2	5	3,330	507	15.2	5	2,870	279	9.74
	1+2								
Pyrocatechol	1	1	12,100			1	12,100		
	2	1	12,100			1	12,100		
Resorcinol	1	5	2,710	664	24.5	5	3,460	597	17.2
	2	5	2,870	279	9.74	5	3,110	560	18.0
	1+2								
Hydroquinone	1	5	16,600	2,470	14.9	5	14,200	1,420	10.0
	2	5	20,600	3,170	15.3	5	19,100	2,480	13.0
	1+2								
Pyrogallol	1	5	12,800	1,990	15.5	5	11,900	1,200	10.1
	2	5	10,400	2,100	20.2	5	9,600	1,430	14.9
	1+2								
Phloroglucinol	1	5	1,030	100	9.76	5	1,030	100	9.76
	2	5	886	68.4	7.71	5	960	143	14.9
	1+2								
o-Nitrophenol	1	5	755	201	26.6	5	739	56.8	7.68
	2	5	551	47.9	8.67	5	499	86.2	17.3
	1+2								
m-Nitrophenol	1	5	222	34.7	15.6	5	228	0	0
	2	5	266	41.4	15.5	5	257	44.3	17.2
	1+2								
p-Nitrophenol	1	5	1,640	143	8.70	5	1,620	393	24.3
	2	5	1,920	350	18.2	5	1,910	248	13.0
	1+2								
2.4-Dinitrophenol	1	5	800	119	14.9	5	778	159	20.4
	2	5	620	96.8	15.6	5	616	47.9	7.77
	1+2								

data obtained on testing observers in group I with actual compounds under the conditions given.

the epithelial surfaces in the mouth (see pp. 453–456). Abbreviations: $\bar{X}$ = arithmetical mean, s = standard thresholds), b = between, i = interaction, O = observers, W = qualities of water and T = temperatures. columns for source of variation are explained on p. 494.

11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
									Source of variation						
	Tap-water														
	Temp. 27°C			Temp. 35°C											
n	$\bar{X} \times 10^6 M$	$s \times 10^6 M$	C.V.	n	$\bar{X} \times 10^6 M$	$s \times 10^6 M$	C.V.	Factorial used	b.O	b.W	i.O × W	b.T	i.O × T	i.W × T	i.O × W × T
5	221	21.9	9.91	5	177	18.1	10.2	2 × 2	—	*	—		—		—
5	238	30.4	12.8	5	192	30.2	15.7	2 × 2	—	*	—		—		—
								2 × 2 × 2		**		*		*	
5	89.2	13.9	15.6	5	83.0	12.7	15.3	2 × 2	—		—		—		—
5	96.0	14.3	14.9	5	95.4	8.18	8.58	2 × 2	—	**	—		—		—
								2 × 2 × 2		***					
5	66.8	11.9	17.9	5	69.5	25.5	36.6	2 × 2	—		—		—		—
5	62.8	14.5	23.1	5	61.8	5.92	9.56	2 × 2	—		—		—		—
								2 × 2 × 2							
5	68.8	6.96	10.1	5	64.5	11.7	18.2	2 × 2	—	*	—		—		—
5	57.3	5.86	10.2	5	46.0	3.94	8.56	2 × 2	—		—	**	—	*	—
								2 × 2 × 2	***	*		**			
5	15.3	1.16	7.60	5	12.8	0.984	7.71	2 × 2	—		—	*	—		—
5	12.5	2.51	20.1	5	9.60	1.43	14.9	2 × 2	—		—		—		—
								2 × 2 × 2	**					*	
5	4,840	1,030	21.3	5	4,300	670	15.6	2 × 2	—	***	—		—		—
5	4,660	1,010	21.6	5	3,560	273	7.66	2 × 2	—	**	—	**	—		—
								2 × 2 × 2		***		**			
1	12,100			1	14,500										
1	12,100			1	14,500										
5	4,150	717	17.3	5	3,710	479	12.9	2 × 2	—	**	—		—	*	—
5	4,420	0	0	5	3,680	0	0	2 × 2	—	***	—		—	**	—
								2 × 2 × 2		***				**	
5	14,800	1,420	9.64	5	15,300	1,160	7.60	2 × 2	—		—		—		—
5	16,600	2,470	14.9	5	17,100	1,750	10.3	2 × 2	—	*	—		—		—
								2 × 2 × 2	***	*					
5	15,500	3,180	20.5	5	14,200	1,420	10.0	2 × 2	—	*	—		—		—
5	12,800	1,990	15.5	5	14,200	1,420	10.0	2 × 2	—	***	—		—		—
								2 × 2 × 2	**	***					
5	1,010	242	23.9	5	1,070	167	15.6	2 × 2	—		—		—		—
5	1,060	81.8	7.69	5	1,150	171	14.9	2 × 2	—	**	—		—		—
								2 × 2 × 2		*					
5	825	83.8	10.2	5	825	83.8	10.2	2 × 2	—		—		—		—
5	620	96.8	15.6	5	555	82.9	14.9	2 × 2	—		—		—		—
								2 × 2 × 2	***	*					
5	300	63.6	21.2	5	199	29.7	14.9	2 × 2	—		—	*	—	**	—
5	317	24.1	7.61	5	265	20.6	7.76	2 × 2	—		—		—		—
								2 × 2 × 2	**	*		**		**	
5	1,480	142	9.64	5	1,420	142	10.0	2 × 2	—		—		—		—
5	1,850	290	15.7	5	1,480	142	9.64	2 × 2	—	*	—		—		—
								2 × 2 × 2	**	**					
5	744	116	15.6	5	697	148	21.3	2 × 2	—		—		—		—
5	602	122	20.3	5	666	99.2	14.9	2 × 2	—		—		—		—
								2 × 2 × 2	**						

Table 3 (*continued*

1	2	3	4	5	6	7	8	9	10
2.5-Dinitrophenol	1	1	2,740						
	2	1	2,740						
2.6-Dinitrophenol	1	1	838						
	2	2	262						
2.4.6-Trinitrophenol	1	5	2.01	0.345	17.2	5	2.11	0.559	26.5
	2	5	2.18	0.528	24.3	5	2.08	0.322	15.5
	1+2								
4-Nitroresorcinol	1					5	1,110	202	18.1
	2					5	1,110	202	18.1
Quinine hydrochloride	1	5	2.75	0.422	15.3	5	2.54	0.252	9.90
	2	5	1.98	0.370	18.7	5	1.70	0.259	15.2
	1+2								
Phenylthiourea	1	5	4.48	0.811	18.1	5	4.77	0.482	10.1
	2	5	3.83	0.331	8.64	5	3.58	0.558	15.6
	1+2								
Sucrose	1	5	8,300	1,270	15.3	5	7,950	684	8.61
	2	5	12,300	1,200	9.78	5	9,900	1,000	10.1
	1+2								

Table 4. Assessments made by observers in group II on series of concentrations

The class mean represents the arithmetical mean of two neighbouring concentrations tasted.
The meaning of the superscripts in cols. 3a–22b

1	2	3 a	3 b	4 a	4 b	5 a	5 b	6 a	6 b	7 a	7 b	8 a	8 b	9 a	9 b	10 a	10 b	11 a	11 b	12 a	12 b
Concentrations of solutions tasted, $\times 10^3$ M	Class mean, $\times 10^3$ M	Observers B. A. Series 1, 2		B. E. Series 1, 2		G. E. Series 1, 2		L. E. Series 1, 2		K. F. Series 1, 2		A. G. Series 1, 2		G. H. Series 1, 2		B. L. Series 1, 2		A. M. Series 1, 2		E. M Series 1, 2	
16.0		1^6	1^5	1^1	1^7	1^6	1^6	1^1	1^4	1^3	1^2	1^4	1^2	1^6	1^1	1^3	1^6	1^1	1^4	1^6	$1^$
	12.0																				
8.00		1^4	1^2	1^2	1^4	0^1	1^5	1^5	1^3	1^2	1^6	1^3	0^1	1^1	1^6	1^7	1^7	1^4	1^2	1^4	$1^$
	6.00																				
4.00		1^2	1^1	0^3	0^5	0^7	0^7	1^4	1^1	0^1	0^3	0^7	1^5	0^3	0^2	0^1	0^3	1^3	1^6	1^1	$1^$
	3.00																				
2.00		1^5	1^4	0^4	0^2	0^2	0^4	0^2	0^6	0^7	0^7	0^5	0^4	0^4	0^5	0^6	0^1	0^7	0^1	0^7	$0^$
	1.50																				
1.00		0^1	0^3	0^6	0^1	1^3	0^3	0^7	0^7	0^4	0^5	0^1	0^6	0^7	0^3	0^4	0^4	0^2	0^7	0^5	$0^$
	0.750																				
0.500		0^3	0^6	0^7	0^3	0^5	0^1	0^3	0^5	0^5	0^1	0^2	0^7	0^2	0^4	0^5	0^2	0^5	0^3	1^3	$0^$
0 (control)		0^7	0^7	0^5	0^6	0^4	0^2	0^6	0^2	0^6	0^4	0^6	0^3	0^5	1^7	0^2	0^5	0^6	0^5	0^2	$0^$

11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
5	2,760	354	12.9	5	2,300	298	13.0								
5	2,560	252	9.86	5	2,300	298	13.0								
5	705	88.4	12.5	5	655	19.1	2.91								
5	240	30.9	12.9	5	232	35.9	15.5								
5	2.89	0.496	17.2	5	2.59	0.470	18.1	2×2	—	**	—		—		—
5	3.36	0.713	21.2	5	2.99	0.463	15.5	2×2	—	***	—		—		—
								2×2×2		***					
				5	1,980	170	8.60								
				5	1,720	263	15.3								
5	3.71	0.799	21.5	5	4.10	0.539	13.1	2×2	—	***	—		—		—
5	2.75	0.422	15.3	5	3.54	0.532	15.0	2×2	—	***	—		—	**	—
								2×2×2	***	***				**	
5	5.73	0.586	10.2	5	3.83	0.331	8.64	2×2	—		—	**	—	***	—
5	3.73	0.676	18.1	5	3.11	0.560	18.0	2×2	—		—		—		—
								2×2×2	***			**		**	
5	8,670	1,750	20.2	5	7,440	1,160	15.6	2×2	—		—		—		—
5	11,100	1,430	12.9	5	9,600	1,430	14.9	2×2	—		—	**	—		—
								2×2×2	***			**			

of resorcinol dissolved in "distilled" water which was also used for rinsing.

The presence or absence of taste in a solution is marked by the figure 1 or 0 respectively. is explained in the text on p. 448.

13 a	13 b	14 a	14 b	15 a	15 b	16 a	16 b	17 a	17 b	18 a	18 b	19 a	19 b	20 a	20 b	21 a	21 b	22 a	22 b	23	24	25
J.P.		S.S.		H.S.		I.S.		O.S.		E.S.		B.S.		M.T.		B.W.		B.Ö-S.		Thresholds per class mean		
Series 1,	2	Series 1,	2	Series 1,	2	Series 1,	2	Series 1,	2	Series 1,	2	Series 1,	2	Series 1,	2	Series 1,	2	Series 1,	2	Series 1	Series 2	Series 1+2
1^5	1^5	1^5	1^7	1^3	1^5	1^3	1^4	1^4	1^6	1^7	1^3	1^1	1^3	1^4	1^4	1^3	1^2	1^2	1^1			
1^3	1^1	1^7	1^5	1^5	1^4	1^1	1^6	1^6	1^3	1^3	1^2	1^3	1^6	1^3	1^2	1^6	1^1	1^3	1^5	1	1	2
0^2	0^2	0^1	0^6	1^1	1^3	1^2	1^2	1^5	1^1	1^6	1^1	0^6	0^5	0^7	0^3	1^7	1^7	1^1	1^4	9	9	18
0^4	0^6	0^4	0^1	1^7	1^1	0^5	0^3	1^2	1^4	0^2	0^4	0^4	0^4	0^5	0^5	0^1	0^5	0^4	0^6	7	7	14
0^6	0^4	0^3	0^4	1^6	0^2	0^6	1^7	0^7	0^5	0^5	0^6	0^2	0^2	0^6	0^1	0^2	0^6	0^7	0^2	2	3	5
0^1	0^7	0^6	0^3	0^4	0^7	0^7	0^5	0^3	0^2	0^4	0^5	0^7	0^1	0^2	0^7	0^5	0^3	0^5	0^7	1		1
0^7	0^3	0^2	0^2	0^2	0^6	1^4	0^1	0^1	0^7	0^1	0^7	0^5	0^7	0^1	0^6	0^4	0^4	0^6	0^3			

during the experiment was greater than one step in the concentration series. On this account the assessments have been considered correct. This line of thought forces us in practice to the conclusion that in one and the same determination of the threshold two values for the absolute threshold have been obtained. In the subsequent elaboration of the results, either the arithmetical mean of the two values or both values can be chosen as representing the series. In the first alternative we may say that the threshold has been determined with greater precision, since we are able to indicate a spreading around the mean value. In principle this may, however, affect the statistical elaboration of the material, since in this case the variables are not equivalent from the statistical point of view.

The first value obtained for the threshold has been chosen as representative of a series that had been assessed in the above-mentioned way.

The threshold values obtained under each of the given conditions, viz. distilled water at 20.4° and 36.9°C and tap-water at 20.4° and 36.9°C, respectively, have been expressed (Table 3) as the arithmetical means of the actual variables. The standard deviation (s) and the coefficient of variation in percentage form ($C.V. = s \cdot 100/\bar{X}\,\%$) are also presented.

Group II as a whole

As has already been mentioned, the standardized test method was also used with 20 persons, of whom 18 had no previous experience in tasting experiments (see p. 438), while two had had almost daily training for a considerable time. Thus the group was heterogeneous in this respect. The untrained observers were given detailed instructions about the methods of the experiments and for two days before the beginning of the experiment were required to taste and assess racks on different occasions. These racks contained concentration series of quinine hydrochloride and sucrose respectively. These preliminary experiments showed that a geometrical series of concentrations, in which each concentration was twice as strong as the immediately preceding concentration, as a rule also straddled the absolute thresholds of all observers. Each of these thresholds was, however, only roughly estimated. The type of concentration series described was afterwards used in all determinations of thresholds. For each determination of threshold the author chose the span of concentrations suitable for the investigation, basing his choice upon the threshold values determined for the two observers in group I. The two concentrations in the middle of the new series then included the above-mentioned threshold values.

Resorcinol was the first substance tested during test period A (Table 1, p. 436, col. 10). The result of this threshold determination is given in Table 4. Since the determination of the threshold for each of the 20 observers was carried out, on the whole, in the same way as that in group I, the material obtained can in principle be dealt with in the same way. For this reason, in the following description only supplementary details are given. Otherwise the reader is referred to the commentaries in connection with Table 2 (p. 443).

Col. 1 in Table 4 contains the concentrations in the geometrical series tasted. The assessment by the observers of each concentration offered is given in cols. $3a$–$22b$. Superscripts near a 1 or a 0 indicate the order in which the liquid in question was judged in the series.

In rare instances the two stronger of three neighbouring concentrations (col. $12a$) or even the three stronger of four neighbouring weak concentrations (col. $5a$) were

assessed as 0, while the weakest was given a 1. There were also cases in which the weaker of two neighbouring concentrations was marked with 1, while the stronger was given a 0 (cf. cols. 8*b* and 16*b*). As the frequency of such a series was about the same as the number of erroneous assessments of the control sample, viz. *c.* 3–4 cases out of 100, I have assumed that, in assessing the lowest concentration which had been given the figure 1 in the series in question, the observer had mistaken irrelevant sensory perceptions for taste sensations. To judge from the experience acquired during the threshold determinations with two observers it seems improbable that the level of the threshold should have varied by over 100 % in the course of the short time required for the tasting of the concentration series. For this reason, the absolute threshold was taken as being represented by the arithmetical mean of the highest concentration that had been given a 0 and the next following higher concentration that had been marked with the figure 1. Table 4, cols. 23, 24, and 25, give the numbers of thresholds per class mean for series 1 and 2 and also for series 1 + 2.

It sometimes happened that some of the observers marked all liquids with 0 or all of them, with the exception of the control, with the figure 1. In such cases the person in question was required to repeat the entire procedure about 10 minutes after the termination of the assessment of the two series. The concentration series offered this time contained three of the concentrations which had been assessed in the first attempt, together with three new ones which formed a direct continuation of the series in the direction of the value, at which the absolute threshold was expected to lie. Thus the last-mentioned concentrations were tasted only by those observers for whom the threshold in question could not be determined during the first taste experiment.

A complementary determination of a threshold was made in experiments Nos. 2A, 6A, 11A, 13A, 18A, 1C, 2C, 10C, and 13C. As has already been mentioned (p. 439), the threshold for each substance was determined twice in immediate succession for the 20 observers forming group II.

Table 5, cols. 4 and 9, contains the mean values expressed as the arithmetical means of the $2 \times 20 = 40$ values obtained for the threshold in question for all observers in group II, "distilled" water, pH 5.1 and 7.8, respectively. In the case in which not *all* of the observers stated the same taste quality (see p. 485), the mean threshold value of the group of only those observers who reported one and the same taste quality is also presented in the same columns. The deviation, *d*, between the mean value of the first and second thresholds of group II and each of these values is given in cols. 5 and 10.

IV. Conditions in the oral cavity during the determination of the thresholds

A. *The effect of rinsing*

The discharge of a solution only incompletely removes the stimulus from the oral cavity. This may be thought to influence the assessment by the observers of the subsequent sample in the following two ways:

1. The remaining quantity of the substance in the oral cavity is added to the amount of the substance in the following sample (effect of addition). If thereby the concentration of the tasted substance reaches the level of the threshold, the estimated value of the threshold will be lower than the "real" one.

Table 5. Statistics calculated and results of *t*-tests performed on threshold data obtained on testing observers in group II with actual compounds under the experimental conditions given.

Abbreviations: $\bar{X}_{1+2}$ = arithmetical mean of first and second mean threshold values for the whole group or the actual part of the group considered, d = deviation from the arithmetical mean ($\bar{X}_{1+2}$) of the first and second mean threshold values obtained for the compound in question, n = total number of threshold data used in the calculations. The digits behind and in front of the sign of multiplication refer to the number of thresholds estimated for each observer and the number of observers respectively. The symbols *, ** and no asterisk in the col. for the *t*-test are explained on p. 491.

1	2	3	4	5	6	7	8	9	10	11	12
	pH of solution tasted = 5.1					pH of solution tasted = 7.8					
	Expt. No.	n	$\bar{X}_{1+2} \times 10^6$ M	$d \times 10^6$M	$\bar{X}_{1+2}$, named	Expt. No.	n	$\bar{X}_{1+2} \times 10^6$ M	$d \times 10^6$ M	$\bar{X}_{1+2}$, named	t-value obtained
Benzene	A21	See p. 438									
Nitrobenzene	A11	20 × 2	390	7	Sweet	C13	20 × 2	424	19	Sweet	0.722
1.2-Dinitrobenzene	A13	20 × 2	175	7	Sweet	C16	20 × 2	219	9	Sweet	2.382*
1.3-Dinitrobenzene	A3	20 × 2	102	10	Bitter	C14	20 × 2	146	4	Bitter	2.744*
1.4-Dinitrobenzene	A6	20 × 2	112	6	Bitter	C15	20 × 2	165	5	Bitter	2.680*
1.3.5-Trinitrobenzene	A10	20 × 2	31.9	1.2	Bitter	C10	20 × 2	42.8	3.6	Bitter	1.109
Phenol	A14	20 × 2	6,110	40	Sweet	C8	20 × 2	6,900	40	Sweet	0.092
Pyrocatechol	A21	See p. 438									
Resorcinol	A1	20 × 2	4,560	20	Sweet	C3	20 × 2	5,150	390	Sweet	1.037
	Replicate 1	20 × 2	5,210	190	Sweet						
	Replicate 2	20 × 2	5,700	80	Sweet						
	Replicate 3	20 × 2	4,850	200	Sweet						
	Replicate 4	20 × 2	5,350	40	Sweet						
	Replicate 5	20 × 2	5,210	230	Mixed						
		18 × 2	5,080	240	Sweet						
Hydroquinone	A8	20 × 2	15,600	1,100	Sweet	C5	20 × 2	12,400	200	Mixed	1.955
							19 × 2	12,600	200	Sweet	
Pyrogallol	A5	20 × 2	17,100	1,900	Bitter	C4	20 × 2	14,200	100	Bitter	0.160
		20 × 2	12,700	700	Sweet taste at rinsing		20 × 2	12,300	1,200	Sweet taste at rinsing	0.173
Phloroglucinol	A17	20 × 2	1,430	90	Sweet	C12	20 × 2	1,590	40	Sweet	0.925

p-Nitrophenol	A12	20 × 2	3,150	110	Sweet	C7	20 × 2	2,360	130	Sweet	2.897 **
2.4-Dinitrophenol	A20	20 × 2	1,250	80	Sweet	C9	20 × 2	905	23	Sweet	1.450
2.5-Dinitrophenol	A19	20 × 2	1,570	100	Sweet	C2	20 × 2	1,290	20	Mixed	1.064
							18 × 2	1,400	30	Sweet	
2.6-Dinitrophenol	A21	See p. 438									
2.4.6-Trinitrophenol	A4	20 × 2	3.60	0.19	Bitter	C1	20 × 2	8.08	0.09	Bitter	2.625 *
Benzaldehyde	A21	See p. 438									
Vanillin	A21	See p. 438									
Veratrole	A21	See p. 438									
Quinine hydrochloride	A15	20 × 2	4.02	0.04	Bitter						
Phenylthiourea	A2	20 × 2	82.4	0.20	Bitter						
		18 × 2	15.9	0.20	Bitter						
		2 × 2	682	0	Bitter						
Sucrose	A16	20 × 2	15,700	300	Sweet						
Sodium chloride	A18	20 × 2	15,400	1,000	Saline						
Sodium nitrite	B2	20 × 2	3,020	200	Mixed						
		19 × 2	3,050	210	Sweet						
Potassium nitrite	B3	20 × 2	3,050	170	Mixed						
		18 × 2	3,120	180	Sweet						
Nitromethane	B9	20 × 2	159,000	2,000	Sweet						
Nitroethane	B7	20 × 2	46,100	1,100	Sweet						
1-Nitropropane	B6	20 × 2	10,600	600	Mixed						
		20 × 2	18,700	300	Sweet						
2-Nitropropane	B1	20 × 2	20,600	1,200	Mixed						
		14 × 2	17,400	1,200	Bitter						
Methanol	B5	20 × 2	1,180,000	40,000	Mixed						
		20 × 2	2,330,000	150,000	Sweet						
		20 × 2	1,680,000	40,000	Bitter						
Ethanol	B4	20 × 2	856,000	23,000	Mixed						
		20 × 2	2,300,000	130,000	Sweet						
		20 × 2	1,020,000	60,000	Bitter						
2-Propanol	B8	20 × 2	410,000	12,000	Mixed						
		19 × 2	486,000	67,000	Bitter						
		14 × 2	1,130,000	10,000	Sweet						

Table 6. Data obtained in studying the effects of rinsing on observers in groups I and II.

Abbreviations: n = number of actual rinsings performed, $\bar{X}_{370nm}$ and $\bar{X}_{600nm}$ = arithmetical mean of extinction of tasted and discharged rinsings at wavelengths 370 and 600 nm respectively, $R\%$ = the proportion (in %) between the concentrations of *o*-nitrophenol in the discharged rinsings and in the original solution tasted.

1	2		3		4		5		6		7	
	n Group		$\bar{X}_{370\,nm}$ Group		$\bar{X}_{600\,nm}$ Group		Calculated extinction of saliva at 370 nm Group		Calculated extinction of *o*-nitrophenol at 370 nm Group		$R\%$ Group	
	I	II	I	II	I	II	I	II	I	II	I	II
Conc. *o*-nitrophenol solution	5	5	9.5	9.1								
Conc. *o*-nitrophenol solution + saliva	10	20	9.250	8.775	.146	.137	.256	.233	8.994	8.542	95.	94.
Rinsings												
1	10	20	.435	.366	.070	.083	.123	.141	.312	.225	3.3	2.5
2	10	20	.188	.194	.057	.061	.100	.104	.088	.090	.9	1.0
3	10	20	.111	.110	.043	.041	.075	.070	.036	.040	.4	.4
4	10	20	.084	.093	.039	.042	.068	.071	.016	.022	.2	.2
5	10	20	.068	.071	.035	.037	.061	.063	.007	.008	.07	.09
6	10	20	.061	.062	.034	.034	.060	.058	.001	.004	.01	.04
7	10	20	.057	.053	.032	.030	.056	.051	.001	.002	.01	.02
8	10	20	.055	.046	.032	.027	.056	.046	−.001	.000	—	0
9	10	20	.049	.046	.028	.026	.049	.044	.000	.002	0	.02
10	10	20	.044	.043	.025	.024	.044	.041	.000	.002	0	.02
11		20		.042		.025		.043		−.001		—
12		20		.041		.024		.041		.000		0
13		20		.042		.024		.041		.001		.01
14		20		.039		.023		.039		.000		0
15		20		.039		.024		.039		.000		0

2. The remaining substance in the oral cavity may, on the other hand, exert an adapting influence upon the taste receptors, with the result that the observer does not perceive a stimulus which slightly exceeds the "real" threshold level (effect of adaptation). In this way the estimated value of the threshold becomes higher than the "real" value (cf. von Békésy (1965)).

The connection has been studied between the concentration of the remaining substance in the oral cavity and the number of rinsings according to the method described. For this purpose each of the observers in group I took in 15 ml of a concentrated solution of *o*-nitrophenol, which they circulated around their mouths for 6 seconds and then discharged. This was followed by 10 rinsings, each with 15 ml of water, according to the method used in the taste experiments. This procedure was repeated five times by each observer at intervals of 20 minutes. Later a similar experiment was carried out with the group of 20 observers, but now the number of rinsings was increased to 15. All discharged liquids were collected and their extinction determined by means of a Beckman Spectrophotometer DU at the wavelength of 370 nm, where the adsorption curve for *o*-nitrophenol has an isosbestic point for

varying pH, and at the wavelength of 600 nm, where the adsorption of the concentrated solution of *o*-nitrophenol in water at pH 5–10 is zero or nearly zero. The arithmetical mean of the extinction obtained is given in Table 6, cols. 3 and 4. Thus the values at 600 nm have been caused by the saliva present in the samples (col. 4). Probably the scattering of the light by the colloidal particles present caused the extinction. Experiments, not described here, have proved the existence of approximate proportionality between the dilution of the saliva and the extinction values obtained. If the ratio between the extinctions of the saliva in the sample in question at the wavelengths of 370 and 600 nm is known, then the contribution of the saliva to the total extinction obtained at 370 nm can be estimated. The ratios between the mean extinctions at 370 and 600 nm in the last five rinsings in the experiments carried out by two observers and the last nine rinsings in the experiments with 20 observers are *c.* 1.75:1 and 1.7:1, respectively. Roughly the same ratio could be established for weakly diluted saliva.

The calculated values for the extinction at 370 nm given in col. 5 for the saliva as a component of the samples have been obtained by multiplying the mean values for the extinction obtained at 600 nm (col. 4) by 1.75 and 1.7, respectively. By finally subtracting the calculated values in col. 5 from the extinction values in col. 3, the calculated value for *o*-nitrophenol is obtained as the remainder (col. 6).

Col. 7 shows that in the fifth rinsing water the concentration amounted to only *c.* 1 % of the original solution of *o*-nitrophenol. Thus in determining the threshold by these methods, a corresponding amount of the substance in the previously assessed liquids may be added to the quantity in the preparation to be assessed after it. From this it is evident that the above-mentioned effect of addition is of small importance. As the concentration of the compound in the rinsing water that precedes a liquid to be assessed must be assumed to be considerably below the threshold, the effect of adaptation probably plays only a very insignificant rôle.

The comparatively low rinsing effect obtained may be due to the fact that part of the substance in the concentrated solution diffuses into the mucus attached to the mucus membrane. Alternatively the substance may be adsorbed into the membrane itself and/or gathered into cavities filled with liquid, where the interchange of liquids is slow.

B. *The temperature conditions at the epithelial surfaces of the oral cavity during the taste experiments*

In the course of the taste experiments the tasted solutions and the rinsing water, prior to their introduction into the oral cavity, had on different occasions temperatures of, on the average, 20.4° and 36.9°C (group I) and 20.8°C (group II), respectively. Unless the temperature of the epithelial surfaces in the oral cavity coincides with that of the liquids taken, changes in temperature can be expected to take place during the rinsings. With the aim of getting an idea of the temperature conditions during the determinations of the thresholds on epithelial surfaces with taste buds, the temperature at the surface of the tongue was continuously recorded in one observer (A. Marcström) in a series of experiments. These experiments resembled the taste experiments in all essential points.

The variations of temperature were recorded in the following way. A copper wire was joined to each end of a constantan wire, so that two thermocouples were obtained. The free ends of the copper wires were connected to a measuring instrument

(Hewlett Packard 425 A microvolt-ammeter) and a recording potentiometer (Varian G 14). One of the two thermocouples was used for reference and was kept during the entire experiment in a thermostat-controlled water-bath at the same temperature as the tasted liquids. The other thermocouple was placed in the centre of one end of a thin helical spring covered with plastic. The other end of the spring was fitted with a plastic suction disk.

Prior to every experiment, the thermocouple upon the spring was immersed in the thermostat-controlled water-bath, so that the automatic recorder drew a zero line upon the paper ribbon. Subsequently the entire spring was introduced into the mouth, the suction disk being pressed against the palate. The other end of the spring rested upon the tongue. In this way the thermocouple came into intimate contact with the surface epithelium of the tongue and the layer of liquid upon it. The recorder then registered the difference in temperature between the surface epithelium and the thermostat-controlled water-bath. The amplitude of the deflection is directly proportionate to the difference of temperature in degrees C within the examined span of temperature. On account of the small mass of the spring and thermocouple these items have been assumed to have had no noteworthy influence upon the changes of temperature in the oral cavity.

In accordance with the methods previously explained, the subject rinsed his mouth with the liquids in four racks—each with 39 rinsings—in succession, the time interval between two series being 3 minutes. In racks Nos. 1, 2, and 4 the rinsing water had a temperature of 20.4°C, while in rack No. 3 its temperature was 36.9°C.

In the course of 2 days altogether six experiments of this kind were carried out. After each of the experiments a respite of at least 1 hour was given.

The curves drawn on the paper ribbon by the recorder give a detailed picture of the changes of temperature at the surface of the tongue during each experiment. From these curves have been read the temperatures of the epithelium immediately after the taking and the discharge, respectively, of each rinsing water. These data for each rinsing have been grouped ($n = 6$), and the arithmetical mean of the group has been calculated.

The mean temperatures calculated in this way for the epithelium of the tongue immediately after the taking (Fig. 1, circles) and at the discharge (Fig. 1, dots) of each rinsing water in the racks show in a rough way the changes of temperature during the tasting of each series. The standard deviation of the measured values has been introduced for every fifth rinsing water.

Since a nose clamp was used during the experiments, the breathed air had to pass the oral cavity in both directions. As a rule all observers exhale in connection with the discharge of a liquid and inhale immediately before taking the next.

As soon as water at 20.4°C is applied to the oral cavity (racks 1, 2, and 4), the temperature at the surface of the epithelium falls at a roughly constant rate up to the time of the discharge. In connection with the following exhalation the temperature starts rising again. At first the rise is fairly fast, but the rate soon slows down or drops to zero at the following inhalation (these portions of the registered curves are not represented in Fig. 1) due to the cooling effect of the inhaled air (temperature of the room *c.* 22°C). During the tasting of the liquids at 20.4°C in each rack the falls and rises of temperature succeed each other essentially in the same way in unison with the rinsings. However, not every fall in temperature is followed by a rise of the same magnitude. This results in a gradual cooling down of the epithelial surface.

The temperature of the epithelial surface of the tongue varies considerably also in

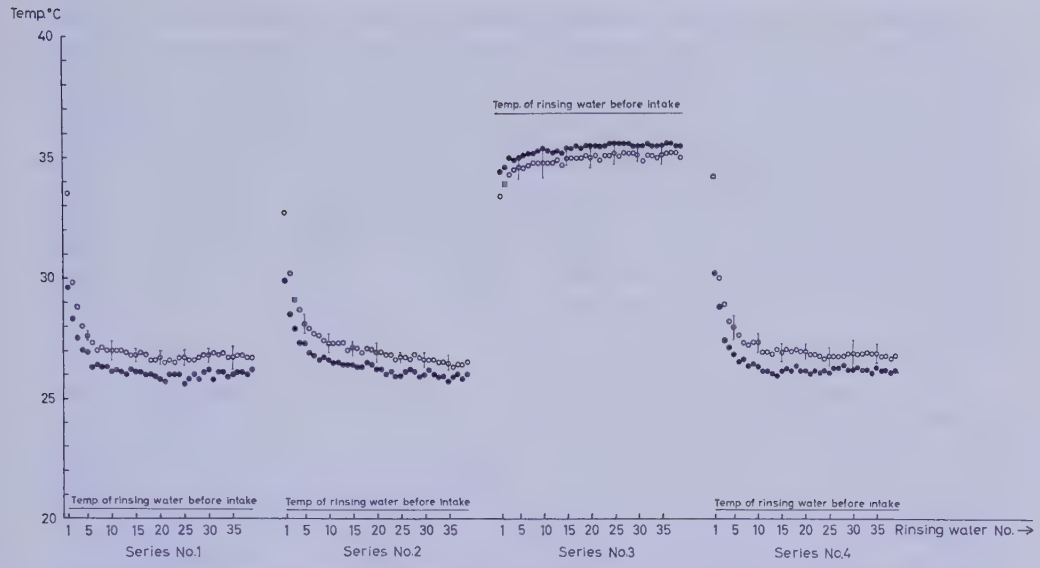

Fig. 1. Effect of rinsing on the temperature at the epithelial surface of the tongue. The circles and dots show the mean temperature at the epithelial surface immediately after the intake and at the discharge of an actual rinsing, respectively, The length of the vertical line through each 5th circle indicates two standard deviations.

the course of repeated rinsings with water at 36.9°C (racks No. 3). In the course of each rinsing the temperature rises at a rather uniform rate up to the time of the discharge of the water. The rise ceases during exhalation and is succeeded by a fall in connection with the inhalation. On account of the fact that this fall in temperature is as a rule smaller than the above-mentioned rise, the average temperature at the epithelial surface rises slowly in the course of the experiment without, however, ever reaching 36.9°C.

In the time interval between two series the epithelial surface reaches a temperature of *c.* 32–34°C. This explains the roughly identical course of the curves for series 1, 2, and 4.

As in the determinations of the thresholds, the liquids were always poured directly upon the tongue. However, experiments, not described here, have shown that the curves follow a similar course also when the rinsing water is applied below or at the side of the tongue. For this reason it has been assumed that the registered curves give a representative picture of the temperature at all epithelial surfaces with taste buds during the experiments.

The assessments made by the observers are based upon the taste sensations which appear while the liquid preparations are inside the mouth. All the observers who took part in the determinations of thresholds were unanimously agreed that the taste sensations appear in the course of the first 3 seconds after the application of a stimulus exceeding the threshold value.

Electrophysiological investigations have in most cases shown that the frequency of impulses in the taste nerves increases almost at the moment of the application of a stimulus to the tongue. In a monkey, for instance, the discharge frequency in

the chorda tympani reaches its maximum within less than a second after the application of a taste substance to the tongue (Zotterman 1959). The time interval in question also seems to be very short in human beings, according to the curves given by Diamant *et al.* (1965, Fig. 2). There are, however, exceptions from this rule. Responses to, for instance, glycerin, glycine and DL-alanine (Halpern (1963), experiments with rat) or to ethanol (Diamant *et al.* (1963), experiments with man and dog, Hellekant (1965*a*, *b*), experiments with cat, dog and rat) build up to peak magnitude slowly. To what extent this phenomenon appears when the compounds used in this investigation are tested on man has not yet been examined. It seems probable, however that the temperature at the epithelial surface immediately after the taking of a liquid (Fig. 1, circles) represents the temperature in the taste cells at the time when these cells are supplying information most intensely about the stimulus present better than the temperature at the discharge of the liquids.

The two assessments that are decisive for the determination of the threshold, viz. the assessment of the weakest concentration that causes a sensation and then that of the strongest concentration which fails to cause a sensation, always fall within the part of the recorded curve limited by rinsings Nos. 5 and 35. This is because the solutions are presented in random order. Within this part of the curves in Fig. 1, the points seem to be fitted by a straight line with a comparatively slight slope. Below, the arithmetical mean of all the 31 temperatures, including those from rinsings Nos. 5 to 35, rounded off to the nearest integer, has been taken as representing the temperature in the taste buds during the determination of thresholds. When before the taking the liquids had an average temperature of 20.4°C (group I) or 20.8°C (group II) and 36.9°C (group I), respectively, the temperature of the taste buds at the decisive moment for the determination of a threshold has been given as 27°C and 35°C, respectively.

Thus it has been assumed that the above-mentioned conditions in the experiments can be reproduced with all observers.

C. *The electrolyte content of the tasted liquids*

I have already mentioned (p. 435) that the determination of the absolute thresholds for the observers in group I was preceded by the adjustment of the pH (to *c.* 5.1) of the liquids made up with distilled water. This was effected by the addition of suitable quantities of HCl or NaOH. When tap-water was used as solvent or for rinsing, no such adjustments were made. In the course of the determination of thresholds the observers in group II were offered preparations containing distilled water, the pH of which had been adjusted in the above way to *c.* 5.1 or *c.* 7.8 (see p. 438).

The liquids differ considerably, both as regards concentration and components, from the saliva which normally surrounds the receptors. This has to be kept in mind in evaluating the results obtained. Of particular interest in this case is the difference in concentration between the saliva and the solutions tested with regard to the ions of calcium, sodium, potassium, and chlorine, which are supposed to be of great importance for the maintenance of the potential of the cell membranes (cf. Eccles 1959, Stämpfli 1956). The apical parts of the taste cells are freely accessible to the fluid in the oral cavity by way of the taste pores.

Table 7. Main constituents of Uppsala tap-water supplied from the water tower.

Where no units are given, the quantities are expressed in mg/l. The analyses for Dec. 1959 (col. 2) and Dec. 1960 (col. 4) were carried out at the Institute of Public Health in Stockholm and at the Laboratory of the Uppsala Water Works in accordance with the instructions published by the National Board of Health (Meddelande från Kungl. Medicinalstyrelsen, nr. 87, 1950). The data for Feb. 1965 (col. 5) were obtained from Fil. lic. Torsten Ahl, of the Institute of Limnology, and those for the electrolytic conductivity (col. 3) from Höglund (1961). In March 1960 the Central Analytical Laboratory of the University of Uppsala determined the contents of sodium and chlorine in tap-water drawn at the Institute of Zoophysiology.

1	2	3	4	5
	Dec. 1959	March 1960	Dec. 1960	Feb. 1965
Physical analyses				
Temp., °C	7.5		8.2	
Colour, mg/l Pt	< 5		< 5	
Turbidity	0		0	
Odour, strength at 40°C	0		0	
Odour, kind	0		0	
Residue after sedimentation for 24 hours	0		0	
Chemical analyses				
$KMnO_4$ consumed	3		5	
Residue on evaporation	—		—	
Residue after ignition	—		387	
Electrolytic conductivity $\varkappa \cdot 10^6$	577	551	554	580
pH	7.6		7.4	
NH_4^+	0.1		< 0.1	
Ca^{2+}	119		112	
Total hardness, German degrees	16.7		15.7	
Na^+	—	22.7	—	23
K^+	—		—	7.7
Fe^{2+}	< 0.1		0.10	
Mn^+	< 0.05		< 0.05	
HCO_3^-	315		292	
Cl^-	31	25.3	25	
SO_4^{2-}	40		35	
NO_3^-	5		14	
NO_2^-	0.04		< 0.01	
PO_4^{3-}	< 0.1		< 0.1	
Bacteriological tests				
Number of colonies per ml on gelatine plates after 48 hours at 20°C	0		0	
Fermentation test in lactose broth at 37°C. The most probable number of *coli aerogenes* bacteria per 100 ml	0		0	

1. *The concentration of* Ca^{++}, Na^+, K^+ *and* Cl^- *ions in tap-water*

Data illustrating the quality of the tap-water in December 1959 and December 1960 are given in Table 7, cols. 2 and 4. The determinations of thresholds with groups I and II were made during the earlier part of 1960.

In Uppsala the tap-water is not chlorinated but is free from smell and bacteria

and is remarkably hard. The quality may be considered as being extraordinarily high and constant. Nevertheless the concentration of the different components varies considerably, as can be seen from the table, from year to year and certainly also, though to a minor extent, from day to day.

For the concentrations of the ions of calcium, chlorine, sodium, and potassium, the order of magnitude has been calculated as 3×10^{-3}, $7\text{–}9 \times 10^{-4}$, 1×10^{-3}, and 2×10^{-4} M, respectively. In making these calculations, I have assumed that the value for the concentration of the potassium ions calculated for 1965 is also representative of the period from December 1959 to December 1960. On the basis of the data given for 1959 and 1960 the total concentration of ions has been assumed to lie between 8.5×10^{-3} M and 9.5×10^{-3} M. Most of the ions are those of calcium and bicarbonate.

2. *The concentration of* Ca^{++}, Na^{+}, K^{+} *and* Cl^{-} *ions in "distilled" water used for rinsing and as solvent for non-protolytes*

The distilled water used as solvent and rinsing water in the testing of the observers in groups I and II was obtained from a glass still, and can be assumed to have had a high and uniform quality throughout the period of the experiments. Its electrolytic conductivity was determined (using a conductivity bridge, Leeds & Northrup type 4886 – S with a conductivity cell, Philips type GM4221) at the beginning and in the middle of the experiments as 2.0 and 2.1×10^{-6} ohm^{-1} cm^{-1}, respectively. In the course of the experiments the pH varied between 5.9 and 6.3.

After titration of the rinsing water and the actual solutions of non-protolytes with HCl to pH 5.1, the concentration of the preparations in question was $10^{-5.1} = 7.94 \times 10^{-6}$ M with regard to Cl^{-}. In preparations with pH 7.8, on the other hand, C_{OH^-} was $10^{-6.2} = 0.631 \times 10^{-6}$ M. On account of the complete dissociation of the added salt (NaOH) the concentration of Na^{+} was the same. In the calculations the presence of carbon dioxide or possible acid-base couples was neglected. The insignificant amounts of Ca^{++} and K^{+} originating from the glass vessels were not taken into account.

3. *The concentration of* Ca^{++}, Na^{+}, K^{+} *and* Cl^{-} *ions in solutions of protolytes*

To adjust the pH of a solution of a protolysing substance to 5.1 or 7.8, it is necessary to add a quantity of HCl or NaOH, depending upon the concentration of the solution and the pK_a value of the substance. Thus, the solutions forming part of the series of concentrations used in the determination of the absolute threshold of a protolyte contain different amounts of the ions of chlorine and sodium. An account of the content of protolytes in all the tasted preparations of this kind would, however, be too extensive. For this reason I have limited myself in the following account to the calculated concentrations of the ions of sodium and chlorine in solutions which had pHs of 5.1 or 7.8 and which at the same time, with regard to their content of protolysing substance, represented the absolute thresholds of the observers. In cases in which no threshold value was found, the strongest concentration tested was considered in the same way.

For the protolytes examined, the following equation has been assumed to be approximately valid:

$$\frac{C_{H_3O^+} \times C_{B^-}}{C_{BH}} = K_a, \qquad (1)$$

Table 8. Formulae used in the calculations of the Na- or Cl-ion concentration.

The formulae refer to solutions containing a concentration (C) of an actual protolysing compound, which represents the mean threshold values (or if no such value is obtained, the highest concentration tested) of (1) observers in group I at (*a*) pH 5.1 and 20.4°C and at (*b*) pH 5.1 and 36.9°C and (2) group II at (*a*) pH 5.1 and 20.8°C and at (*b*) pH 7.8 and 20.8°C, the pH and temperature indicating the conditions prevailing in the solutions tested. The subscripts adjoining the crosses refer to the cols. in question in Table 9, in which the results of the calculations will be found. The pK_a value for vanillin is according to Long, C., *Biochemists' Handbook*, E. & F. N. Spon Ltd, London, 1961; for resorcinol and 2.4.6-trinitrophenol according to Weast, R. C., Selby, S. M. and Hodgman, Ch. D., *Handbook of Chemistry and Physics*, 46th ed., Chem. Rubber Publishing Co., Cleveland, 1965; and for the other substances according to Kortüm, G., Vogel, W. and Andrussow, K., *Dissociation constants of organic acids in aqueous solution*, Butterworths, London, 1961. The superscripts after the pK_a values indicate the temperature in °C. If no superscript is given, the reference is to room temperature.

		Formulae													
		No. 3			No. 4				No. 5			No. 6			
		$C_{H_3O^+} = C + C_{Cl^-}$			$pH = pK_a + \log \frac{C_{Na^+} + C_{H_3O^+}}{C - C_{Na^+} - C_{H_3O^+}}$				$pH = pK_a + \log \frac{C_{H_3O^+} - C_{Cl^-}}{C - C_{H_3O^+} + C_{Cl^-}}$			$pH = pK_v + \log(Na^+ - C)$			
		Group I, pH 5.1		Group II, Temp. 20.8°C,	Group I, pH 5.1		Group II, Temp. 20.8°C		Group I, pH 5.1		Group II, Temp. 20.8°C,	Group II, Temp. 20.8°C,			
Substance	pKa	Temp. 20.4°C	Temp. 36.9°C	pH 5.1	Temp. 20.4°C	Temp. 36.9°C	pH 5.1	pH 7.8	Temp. 20.4°C	Temp. 36.9°C	pH 5.1	pH 7.8			
enol	9.98^{25}							X_{21}	X_5	X_{10}	X_{17}				
rocatechol	9.85^{20}								X_5	X_{10}					
sorcinol	9.81^{25}							X_{21}	X_5	X_{10}	X_{17}				
ydroquinone	10.85^{25}							X_{21}	X_5	X_{10}	X_{17}				
rogallol	9.01							X_{21}	X_5	X_{10}	X_{17}				
loroglucinol	8.45							X_{21}	X_5	X_{10}	X_{17}				
Nitrophenol	7.21^{25}							X_{21}	X_5	X_{10}	X_{17}				
Nitrophenol	8.38^{25}							X_{21}	X_5	X_{10}	X_{17}				
Nitrophenol	7.15^{25}				X_4	X_9	X_{16}	X_{21}							
4-Dinitrophenol	4.09^{25}				X_4	X_9	X_{16}	X_{21}							
5-Dinitrophenol	5.22^{25}				X_4		X_{16}	X_{21}							
6-Dinitrophenol	3.71^{25}				X_4										
4.6-Trinitrophenol	0.80^{25}	X_5	X_{10}	X_{17}								X_{24}			
anillin	7.40^{25}				X_4		X_{16}								

where C_{BH} is the concentration of unprotolysed acid in the solution, C_{B^-} the concentration of the base in question, $C_{H_3O^+}$ the concentration of hydronium ions, and K_a the thermodynamic dissociation constant of the acid.

Since it has been assumed that BH and B^- do not take part in other equilibria in the solution, the total amount of added protolyte is

$$C = C_{BH} + C_{B^-}. \tag{2}$$

The equation

$$\sum Z_P \times C_P = \sum Z_N \times C_N, \tag{3}$$

Table 9. Molarities of Na^+, Cl^-, K^+ and Ca^{++} in solutions (before introduction into the mouth), th observers in group I and of group II as a whole—or if n

The pH values and C_B^-/C_{BH} quotients given represent the solutions when tasted. Abbreviations in the tab

1	2	3	4	5	6	7	8	9	10	11	12	13	14

Group I

"Distilled" water

Tap-water

Compound	Observer No.	Mean threshold value or highest conc. tested, $\times 10^6$ M	Concentration in solutions before tasting: $Na^+ \times 10^6$ M	Concentration in solutions before tasting: $Cl^- \times 10^6$ M	Calculated pH of solutions when tasted	Calculated quotient C_B^-/C_{BH} of compound when tasted	Mean threshold value or highest conc. tested, $\times 10^6$ M	Concentration in solutions before tasting: $Na^+ \times 10^6$ M	Concentration in solutions before tasting: $Cl^- \times 10^6$ M	Mean threshold value or highest conc. tested, $\times 10^6$ M	Calculated pH of solutions when tasted	Calculated quotient C_B^-/C_{BH} of compound when tasted	Mean threshold value or highest conc. tested, $\times 10^6$ M
		Temp. 20.4°C					Temp. 36.9°C			Temp. 20.4°C			Temp. 36.9°C
Benzene	1, 2	5,000		7.9	5.9		5,000		7.9	5,000	7.45		5,000
Nitrobenzene	1	174		7.9	5.9		166		7.9	221	7.85		177
	2	168					175			238			192
1.2-Dinitrobenzene	1	72.2		7.9	5.9		76.9		7.9	89.2	7.90		83.2
	2	77.4					79.5			96.0			95.4
1.3-Dinitrobenzene	1	61.8		7.9	5.9		76.1		7.9	66.8	7.30		69.5
	2	68.7					59.9			62.8			61.8
1.4-Dinitrobenzene	1	62.3		7.9	5.9		51.2		7.9	68.8	7.95		64.5
	2	51.2					49.5			57.3			46.0
1.3.5-Trinitrobenzene	1	14.2		7.9	5.9		13.8		7.9	15.3	7.35		12.8
	2	12.0					13.4			12.5			9.60
Phenol	1	3,210		7.9	5.9	8.3×10^{-5}	2,970		7.9	4,840	7.45	3.0×10^{-3}	4,300
	2	3,330					2,870			4,660			3,560
Pyrocatechol	1, 2	12,100		7.7	5.9	1.1×10^{-4}	12,100		7.7	12,100	7.45	4.0×10^{-3}	14,500
Resorcinol	1	2,710		7.9	5.9	1.2×10^{-4}	3,460		7.9	4,150	7.30	3.1×10^{-3}	3,710
	2	2,870					3,110			4,420			3,680
Hydroquinone	1	16,600		7.9	5.9	1.1×10^{-5}	14,200		7.9	14,800	7.45	4.0×10^{-4}	15,300
	2	20,600					19,100			16,600			17,100
Pyrogallol	1	12,800		6.4	5.9	7.8×10^{-4}	11,900		6.5	15,500	7.30	1.9×10^{-2}	14,200
	2	10,400		6.7			9,600		6.8	12,800			
Phloroglucinol	1	1,030		7.5	5.9	2.8×10^{-3}	1,030		7.5	1,010	7.35	7.9×10^{-2}	1,070
	2	886					960			1,060			1,150
o-Nitrophenol	1	755		2.1	5.7	3.1×10^{-2}	739		2.3	825	7.30	1.2	825
	2	551		3.7			499		4.1	620			555
m-Nitrophenol	1	222		7.8	5.9	3.3×10^{-3}	228		7.8	300	7.45	1.2×10^{-1}	199
	2	266					257			317			265
p-Nitrophenol	1	1,640	6.5		5.6	2.8×10^{-2}	1,620	6.4		1,480	7.35	1.6	1,420
	2	1,920	9.0				1,910	8.9		1,850			1,480

ncentrations of which, as regards the compounds tested, represent the mean threshold values of ch values are obtained, the highest concentrations tasted.

e explained in the text. Superscript [1] and [2] in cols. 24 and 26 refer to observer No. 1 and No. 2, respectively.

15	16	17	18	19	20	21	22	23	24	25	26	27
			Group II "Distilled" water						Calculated ratio between the concentration of Na^+ in tasted solutions made up with:		Calculated difference between pH of tasted solutions made up with:	
			pH 5.1				pH 7.8					
Compound	Mean threshold value or highest conc. tested, $\times 10^6$ M	Concentration in solutions before tasting: $Na^+ \times 10^6$ M	Concentration in solutions before tasting: $Cl^- \times 10^6$ M	Calculated pH of solutions when tasted	Calculated quotient C_B^-/C_{BH} of compounds when tasted	Mean threshold value or highest conc. tested, $\times 10^6$ M	Concentration of Na^+ in solutions before tasting, $\times 10^6$ M	Calculated pH of solutions when tasted	Calculated quotient C_B^-/C_{BH} of compound when tasted	"Distilled" water and tap-water, group I	"Distilled" water, pH 5.1 and 7.8, group II	"Distilled" water and tap-water, group I	"Distilled" water, pH 5.1 and 7.8, group II
enzene	5,000		7.9	5.9						1:4.3		1.55	
itrobenzene	390		7.9	5.9		424	0.63	7.0		1:4.3	1:1.0	1.95	1.1
2-Dinitro-enzene	175		7.9	5.9		219	0.63	7.0		1:4.3	1:1.0	2.00	1.1
3-Dinitro-enzene	102		7.9	5.9		146	0.63	7.0		1:4.3	1:1.0	1.40	1.1
4-Dinitro-enzene	112		7.9	5.9		165	0.63	7.0		1:4.3	1:1.0	2.05	1.1
3.5-Trinitro-enzene	31.9		7.9	5.9		42.8	0.63	7.0		1:4.3	1:1.0	1.45	1.1
henol	6,110		7.9	5.9	8.3×10^{-5}	6,900	45	7.4	2.7×10^{-3}	1:4.3	1:1.2	1.55	1.5
yrocatechol	100,000		6.2	5.9	1.1×10^{-4}					1:4.3		1.55	
esorcinol	4,560		7.9	5.9	1.2×10^{-4}	5,150	50	7.4	3.9×10^{-3}	1:4.3	1:1.2	1.40	1.5
	5,210			5.9	1.2×10^{-4}								
	5,700			5.9	1.2×10^{-4}								
	4,850			5.9	1.2×10^{-4}								
	5,350			5.9	1.2×10^{-4}								
	5,210 / 5,080			5.9	1.2×10^{-4}								
ydro-uinone	15,600		7.9	5.9	1.1×10^{-5}	12,400 / 12,600	11	7.5 / 7.4	4.5×10^{-4} / 3.5×10^{-4}	1:4.3	1:1.0	1.55	1.6 / 1.5
yrogallol	17,100	5.8		5.9	7.8×10^{-4}	14,200	820	7.8	6.2×10^{-2}	1:4.3	1:3.7	1.40	1.9
	12,700	6.4				12,300	710	7.7	4.9×10^{-2}		1:3.4		1.8
hloro-lucinol	1,430		7.3	5.9	2.8×10^{-3}	1,590	290	7.7	1.8×10^{-1}	1:4.3	1:2.0	1.45	1.8
Nitrophenol	1,030		0	5.8	3.9×10^{-2}	1,190	950	7.7	3.1	1:4.3	1:4.2	1.60	1.9
-Nitro-henol	516		7.7	5.9	3.3×10^{-3}	519	110	7.6	1.7×10^{-1}	1:4.3	1:1.4	1.55	1.7
Nitro-henol	3,150	20		5.5	2.2×10^{-2}	2,360	1,900	7.8	4.5	1:4.3	1:7.0	1.75	2.3

Table 9 (*continued*)

1	2	3	4	5	6	7	8	9	10	11	12	13	14
2.4-Dinitrophenol	1	800	720		5.6	$3.2 \times 10^{+1}$	778	700		744	7.35	$1.8 \times 10^{+3}$	697
	2	620	560				616	550		602			666
2.5-Dinitrophenol	1	2,740	1,200		5.2	9.6×10^{-1}				2,760	7.25	$1.1 \times 10^{+2}$	2,300
	2									2,560	7.29	$1.2 \times 10^{+2}$	
2.6-Dinitrophenol	1	838	730		5.8	$1.2 \times 10^{+2}$				705	7.45	$5.5 \times 10^{+3}$	655
	2	262	240		5.9	$1.5 \times 10^{+2}$				240			232
2.4.6-Trinitrophenol	1	2.01		5.9	5.9	$1.3 \times 10^{+5}$	2.11		5.8	2.89	7.35	$3.5 \times 10^{+6}$	2.59
	2	2.18		5.8			2.08		5.9	3.36			2.99
4-Nitroresorcinol	1						1,110		(?)		7.25	(?)	1,980
	2												1,720
Benzaldehyde	1	20,000		7.9	5.9		20,000		7.9	20,000	7.40		20,000
	2												
Vanillin	1	100,000	500		5.3	7.9×10^{-3}							
	2												
Veratrole	1	2,000		7.9	5.9		2,000			2,000	7.35		2,000
	2												
Quinine hydrochloride	1	2.75		7.9	5.9	(?)	2.54		7.9	3.71	7.25	(?)	4.10
	2	1.98					1.70			2.75			3.54
Phenylthiourea	1	4.48		7.9	5.9	(?)	4.77		7.9	5.73	7.85	(?)	3.83
	2	3.83					3.58			3.73			3.11
Sucrose	1	8,300		7.9	5.9		7,950		7.9	8,670	7.35		7,440
	2	12,300					9,900			11,100			9,600
Sodium chloride													
Sodium nitrite													
Potassium nitrite													
Nitromethane													
Nitroethane													
1-Nitropropane													
2-Nitropropane													
Methanol													
Ethanol													
2-Propanol													

where $Z_p \times C_p$ and $Z_N \times C_N$, are the products of charge (Z) and concentration (C), respectively, for an arbitrarily chosen positive or negative kind of ion in the solution, and where the sign $\sum$ signifies that all the products in question are to be added up, represents the condition for electroneutrality. By means of equations (1), (2) and (3), new equations for the calculation of the electrolytic content of the solutions may be established. In order to be able to arrive quickly at approximations and, in so doing, to simplify these equations without at the same time causing a noticeable diminution of accuracy in the calculations, I have plotted C_{BH} and C_{B^-} for each substance as functions of $C_{H_3O^+}$ in a system of logarithmic coordinates, according to the method

	15	16	17	18	19	20	21	22	23	24	25	26	27
4-Dinitro-phenol	1,250	1,100		5.5	$2.6 \times 10^{+1}$	905	900	7.0	$8.1 \times 10^{+2}$	1:1.3[1] 1:1.5[2]	1:0.9	1.75	1.5
5-Dinitro-phenol	1,570	670		5.2	9.6×10^{-1}	1,290 1,400	1,300 1,400	7.0	$6.0 \times 10^{+1}$	1:0.9	1:1.6 1:1.8	2.05[1] 2.09[2]	1.8
6-Dinitro-phenol										1:1.2[1] 1:2.4[2]		1.65[1] 1.55[2]	
4.6-Trinitro-phenol	3.60		4.3	5.9	$1.3 \times 10^{+5}$	8.08	8.7	7.0	$1.6 \times 10^{+6}$	1:4.3	1:1.0	1.45	1.1
Nitrore-orcinol										1:4.3		1.35	
enzal-dehyde	20,000		7.9	5.9						1:4.3		1.50	
anillin	50,000	125		5.5	1.3×10^{-2}								
eratrole	2,000		7.9	5.9						1:4.3		1.45	
uinine hy-drochloride	4.02		7.9	5.9	(?)					1:4.3		1.35	
henyl-hiourea	682 82.4		10 7.9	5.9	(?)					1:4.3		1.95	
ucrose	15,700		7.9	5.9						1:4.3		1.45	
odium hloride	15,400		7.9	5.9									
odium nitrite	3,020 3,050		46	5.9	(?)								
otassium nitrite	3,050 3,120		84	5.8	(?)								
itromethane	159,000	62		6.0	(?)								
itroethane	46,100		3	6.0	(?)								
Nitropro-pane	18,700 10,600		10	6.0	(?)								
Nitopro-pane	20,600 17,400	60		5.9	(?)								
ethanol	2,330,000 1,680,000 1,180,000		7.9	5.9									
thanol	2,300,000 1,020,000 856,000		7.9	5.9									
propanol	410,000 486,000 1,130,000		7.9	5.9									

described by Hägg (1962, p. 57 ff.). In constructing this diagram and in the theoretical calculations, only the first dissociation constant of the protolytes has been taken into account. Table 8 contains a survey of the formula used in the calculations. As the ionic content of the solutions is very low, activity coefficients are supposed to be unity. Only protolytes having a pK_a value lower than 11 have been considered. There is reason to suspect that quinine hydrochloride, phenylthiourea, sodium nitrite, potassium nitrite, nitromethane, nitroethane, 1-nitropropane and 2-nitropropane also take part in dissociation equilibria in water. The pK values have, however, not been encountered in the literature. The actual amounts of Na^+ or Cl^- have

therefore been estimated empirically by titrating to the actual pH the solutions whose concentrations represent a mean threshold value. The results have been presented only with two digits in Table 9, cols. 4, 5, 9, 10, 16, 17, and 21. The actual data represent the ion content of the solutions before the tasting. At the taking, the liquids are, however, mixed with the saliva which has had time to collect in the oral cavity in the interval of 6 seconds allotted to the observer after the discharge of a preceding liquid. Table 50 in Spector's *Handbook of Biological Data* (1956) gives a review of the physical and chemical characteristics of human saliva, both with the salivary glands in unstimulated condition and after their stimulation by chewing of paraffin wax. The latter condition has been considered to represent the experimental conditions obtaining during the taste experiments. The flow of saliva has thus been assumed to be *c.* 1.9 ml/min. or 0.19 ml/6 sec. Since, according to the same source, concentrated saliva contains *c.* 23.9 mEq Na^+, 15.5 mEq Cl^-, 18 mEq K^+, and 2.8 mEq Ca^{++} per litre, the concentration of the ions of sodium, chlorine, potassium, and calcium, respectively, in each liquid should rise upon introduction into the mouth by approximately 300×10^{-6} M, 190×10^{-6} M, 230×10^{-6} M, and 18×10^{-6} M, respectively. In solutions, whose concentrations represent the mean threshold values of observers in group II at pH 5.1 and 7.8, the concentrations of Cl^-, K^+ and Ca^{++} are obviously about the same at the tasting. Some of the actual solutions at pH 7.8, however, have appreciably higher concentrations of Na^+, compared with the corresponding ones at pH 5.1, even after mixing with the saliva upon introduction into the mouth. As Table 9, col. 25, shows this is true of compounds Nos. 11–15 and 17. The ratio between the sodium-ion concentrations in solutions at pH 5.1 and 7.8 is for the remaining compounds about 1:1. On the whole, the sodium-ion concentration is, however, comparatively low.

The solutions, whose concentrations represent the mean threshold values of observers in group I hold, when made up with tap-water, approximately the same concentrations of K^+, a concentration of Cl^- 4–5 times greater, and a concentration of Ca^{++} *c.* 170 times greater than the corresponding solutions prepared with distilled water. The sodium-ion concentration in solutions with threshold concentrations of 2.4-, 2.5- and 2.6-dinitrophenol in "distilled" water is *c.* 3, 5 and 2.3 times greater respectively after the tasting than that in solutions of the remaining compounds. It approaches according to col. 24 the level in the corresponding tap-water preparations.

D. *The pH in the oral cavity during a taste experiment*

During the taking and the tasting, each liquid will become mixed with the saliva that has collected in the oral cavity after the discharge of the immediately preceding sample and with the constantly produced saliva. Since the buffer capacity of the distilled water is very low, the pH will be changed considerably during the tasting in the direction of the pH of the saliva. In the observers in group I the pH of the saliva was determined electrometrically (Radiometer 22r) every morning before the start of the experiments aimed at the determination of threshold values. For the concentration of the hydrogen ions in the saliva, the mean value and the standard deviation have been calculated for the entire test period (24 days) and have then been expressed in pH units. For observer No. 1 the mean value was found to be 6.76, the mean value plus and minus the standard deviation being 6.66 and 6.88. The corresponding figures representing observer No. 2 were, in the same order, 6.83, 6.69 and 7.02.

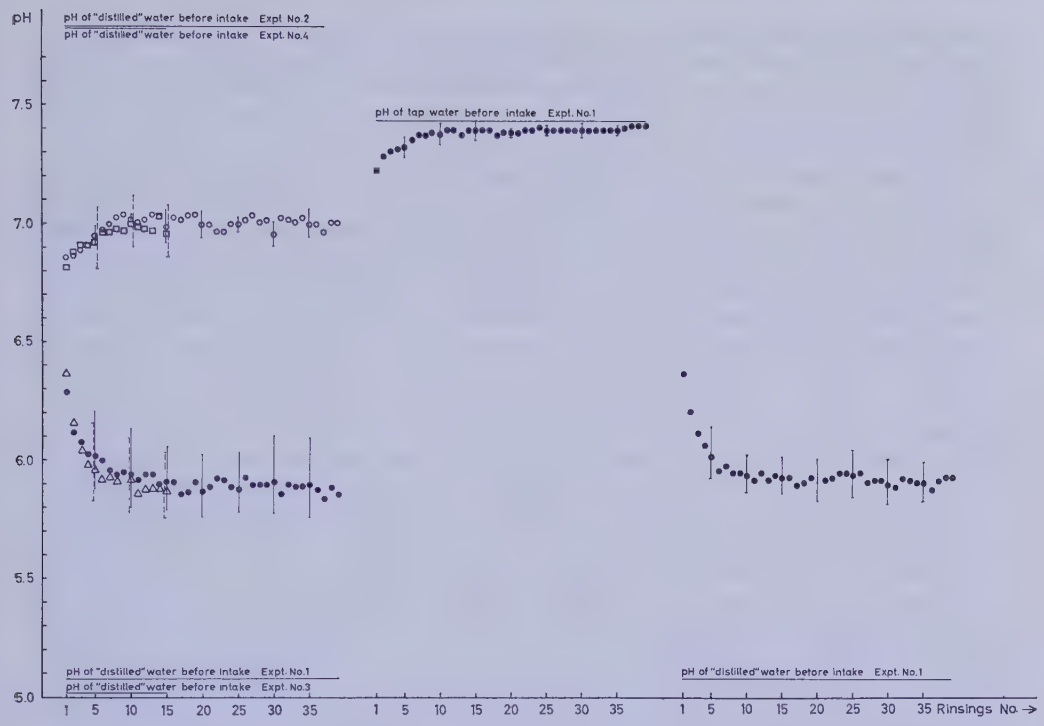
pH
pH of "distilled" water before intake Expt. No.2
pH of "distilled" water before intake Expt. No.4
pH of tap water before intake Expt. No.1
pH of "distilled" water before intake Expt. No.1
pH of "distilled" water before intake Expt. No.3
pH of "distilled" water before intake Expt. No.1
7.5
7.0
6.5
6.0
5.5
5.0
1 5 10 15 20 25 30 35
1 5 10 15 20 25 30 35
1 5 10 15 20 25 30 35 Rinsings No.

Fig. 2. Changes in pH of samples of "distilled" water with different pHs and of tap-water when tasted. Experiment No. 1 with "distilled" water, pH 5.08, and tap-water, pH 7.44 (dots), experiment No. 2 with "distilled" water, pH 7.83 (circles), experiment No. 3 with "distilled" water, pH 5.02 (triangles) and experiment No. 4 with "distilled" water, pH 7.82 (squares). The length of the vertical line through each 5th mean value indicates two standard deviations, each calculated as described in the text.

In order to get an idea of the magnitude of the change in pH in preparations containing non-protolysing substances, I have measured the pH of discharged samples in experiments which in all essential details resembled taste experiments. The experiments described below were carried out with one observer (A. Marcström).

Experiment No. 1. Three racks containing samples of water were tasted in succession, with an interval of *c.* 3 minutes. The first and last rack contained "distilled" water of pH *c.* 5.08, while the second contained tap-water of pH 7.44. The experiment was repeated on six different occasions.

Experiment No. 2. The observer furthermore tasted six racks holding "distilled" water of pH *c.* 7.83. The six racks were tasted at intervals of at least 1 hour. Prior to every experiment, the pH of the saliva was also measured.

The measurements of pH in the discharged preparations were made with a pH-meter of the Radiometer 22r type, which had been connected to a potentiometer recorder (type Varian G14). The samples of water were discharged into an inclined funnel, the spout of which had been bent at an angle, so that the opening faced upwards. Each time about 3 ml of the discharged preparation stayed in the bend. By experiments with intensely coloured preparations it could be shown that at each

discharge the mixture of saliva and water completely replaced the liquid which had previously been found in the bend. The electrodes (glass electrode, type G 200b and calomel electrode, type k 100) were introduced into the narrow opening of the spout. By this arrangement the pH of a tasted preparation was registered by the recorder immediately after the discharge.

The data obtained under *similar* conditions for the concentration of hydrogen ions in saliva, in the rinsing water before the tasting, and in the rinsing waters after discharge have been assembled in groups, each of them containing six variables. For every such group, the arithmetical mean has been calculated and expressed in pH units in Fig. 2. For every fifth rinsing water, vertical lines mark the standard deviations obtained from the standard deviation of the hydrogen-ion concentration. These rinsing waters correspond to the liquids used in the determination of the thresholds.

Experiments Nos. 3 and 4. On two occasions the pH of the saliva was measured in group II. In connection with these experiments the 20 observers were required to taste 15 samples ("distilled" water) with pH 5.02 and pH 7.82, respectively.

The pHs of the rinsing waters discharged by each person were determined. The arithmetical mean and the standard deviation of the concentration of hydrogen ions in the rinsing water in question was calculated for the group and expressed in pH units in Fig. 2 (triangles and squares).

Experiment No. 1 (Fig. 2, dots) shows that during the tasting the pH of the liquid samples is appreciably changed towards that of the saliva. On account of their greater content of saliva, the first discharged rinsing waters exhibit a pH which differs most from that of "distilled" water. The pH in the oral cavity becomes reasonably constant only after seven or eight rinsings. To make the experimental conditions during the tasting of all the assessed preparations in a determination of the thresholds more uniform, the number of rinsings before the taking of the first preparation ought to have been increased to seven or eight.

In order to find out whether or not the pH in the oral cavity is constant if a great number of threshold determinations is carried out in sequence, 10 racks containing "distilled" water at pH *c.* 5.1 were tasted with intervals of *c.* 3 minutes between every two racks. For all racks, the simultaneously registered curves for the pH of the discharged liquids follow a similar course (experiments not described). Also in experiment No. 1 there is very little difference between the curves registered for the first and the third racks, in spite of the fact that the rack tasted between them contained tap-water. The pH in the oral cavity before the tasting of the samples in a rack thus does not exert any essential influence on the conditions during the actual tasting. The measured values obtained at the tasting of samples in the third rack exhibit, however, a slightly smaller standard deviation, presumably due to the fact that on account of the preceding rinsings the ion composition of the saliva before the tasting of the solutions in rack No. 3 had assumed a more uniform character, compared with that existing before the tasting of the samples in the first rack was begun.

According to Fig. 2, the buffer capacity of the tap-water was sufficient to keep the pH during the tasting at almost the same level as before the taking of the preparations. For the same reason the variation of the measured values was very small.

Most probably the changes in the pH of the rinsing water observed during the rinsing with the three types of liquids tested in experiments 1, 2, 3, and 4 give a picture of the conditions during the tasting of solutions with corresponding pHs in the determination of thresholds for non-protolysing compounds. As can be seen from

Fig. 2, experiment No. 1, with "distilled" water pH 5.08, the points seem to gather along a line which is slightly concave upwards in the left-hand part. The portion of the curve of greatest interest as regards the problem in hand is, however, limited by rinsing waters Nos. 5 and 35, since this portion comprises all liquids assessed during the determination of the threshold. Within this portion the curve seems to be rather slightly bent, being furthermore only slightly inclined to the abscissa. It seems therefore that the arithmetical mean of the hydrogen-ion concentration expressed in pH units is representative of all data obtained for the rinsing waters Nos. 5–35 ($n = 6 \times 31 = 186$) for the type of tasted liquids in question. For the discharged liquids from the first rack in experiment No. 1, the mean value and the mean value plus and minus the standard deviation of the hydrogen-ion concentration, converted into pH units, are 5.92, 5.78 and 6.10 respectively. The corresponding values for the liquids from the third rack are, in the same order, 5.93, 5.86 and 6.02. Below, these mean values are rounded off to 5.9. This figure (see Table 9, col. 6) has been taken to represent the pH level in the oral cavity of the observers in group I, when these observers in the course of the determination of thresholds assessed liquids containing unprotolysed substances dissolved in "distilled" water of pH *c.* 5.1. The same applies to the observers in group II under the corresponding conditions (col. 18). In the same way the pH in the oral cavity of the observers in group II has been estimated as *c.* 7.0 (Table 9, col. 22), when solutions of pH 7.8 were assessed. The mean value and the mean value plus and minus the standard deviation of the hydrogen-ion concentration in the actual discharged liquids from the racks tasted in experiment No. 2 (Fig. 2, circles), converted into pH units, were 7.01, 6.95 and 7.08. The reasonableness of the last estimation is also supported by experiments which were carried out with group II and are reported in Fig. 2.

As appears from Table 1, col. 9 p. 437, the alkalinity of the tap-water varied considerably during the test period. For this reason the pH (7.39) representing the arithmetical mean of the hydrogen-ion concentration in the actual 186 discharged liquids from rack No. 2 in experiment No. 1 (the mean value plus and minus the standard deviation being 7.36 and 7.42) does not represent the pH level in the oral cavity of the observers in group I. However, as can be seen also from experiment No. 1 in Fig. 2, the pH of the tap-water drops by approximately 0.05 units during each rinsing of the mouth. This is also considered to be the case in every individual test experiment.

Solutions of protolysing substances, the pK_a values of which are of the order of magnitude 3–11 show, as a rule, even in concentrations representing the threshold for sweet or bitter taste, a buffer capacity which at the pH levels 5.1 and/or 7.8 considerably surpasses that of "distilled" water. With the aim of getting an idea of the degree of shifting of the pHs of such solutions towards that of the saliva, when the solutions are introduced into the mouth for assessment, the following experiment was performed. Observer No. 1 was required to taste, one by one and in the way described above (p. 434), racks each containing 39 samples of liquids with pH 5.1. The 5th, 10th, 15th, 20th, 25th, 30th and 35th preparations contained the substance in question in quantities corresponding to the mean threshold values of observer No. 1, observer No. 2 and group II as a whole respectively. Solutions of pH 7.8 were also tested in the same way. In all the racks all the samples had a temperature of 21.0°C.

The determination of the pH of the discharged preparations was carried out in the way described on p. 465. The arithmetical means were calculated for the given

preparations in each rack. These means have been allowed to represent the pH levels at the epithelial surfaces in the mouths of observers Nos. 1 and 2 and of the observers in group II, when the actual compounds Nos. 7, 9–18, 22, and 28–33 were tasted. The data in question, each with two digits, will be found in Table 9, cols. 6, 12, 18, and 22. In all cases the standard deviation, expressed to one decimal place, is 0.1 pH unit. The values in Table 9, col. 26, represent the differences between the pHs at the epithelial surfaces in the mouths of the observers in group I when the actual solutions containing the compounds dissolved in distilled water and tap-water respectively were tasted. They were obtained as a remainder after subtracting the values found in col. 6 from the corresponding values in col. 12. The differences are within the range 1.35–2.09 units. The value most frequently observed is 1.55.

The values shown in col. 27 in their turn represent the differences between the pHs of the epithelial surfaces in the mouths of the observers in group II when the actual solutions at pH 5.1 and 7.8 respectively were tasted (cf. cols. 18 and 22). The differences are *c.* 1.1 units for non-protolysing compounds and between 1.5 and 2.3 units as regards the remainder.

E. *The quotient between the quantities of protolysed and unprotolysed forms of a substance in the solutions after discharge*

In Table 9, cols. 7, 13, 19, and 23, the quotient C_{B^-}/C_{BH}, i.e. the quotient between the quantities of protolysed (C_{B^-}) and unprotolysed (C_{BH}) forms in the solution, has been calculated for the compounds mentioned in the preceding chapter in the cases in which their pK_a values could be found in the literature. In so doing, I have introduced into the equation $pH - pK_a - \log C_{B^-}/C_{BH}$ the empirically determined pH values, presented in the preceding chapter, for the discharged liquids containing the substance in a concentration representing the absolute threshold. As can be seen, the ionic activity has not been taken into consideration (dilute solutions). The calculations have been carried out for each of the observers in group I and for group II.

As can be seen in Table 9, most of the compounds appear mainly in the unprotolysed form at the actual pH levels studied. However, the quotient C_{B^-}/C_{BH}, representing the compounds *o*- and *p*-nitrophenol and 2.5-dinitrophenol, becomes greater than 1 at the higher pH level tested. Only 2.4- and 2.6-dinitrophenol and 2.4.6-trinitrophenol are almost completely dissociated under the pH conditions prevailing in the mouths of the observers during the experiments in question.

V. Discussion of the methods used

A. *The relationship between the standard deviation of the absolute thresholds of observers in group I and the constancy of the experimental conditions at the surface of the tongue*

On the basis of the experiments described on p. 453 there is reason to assume that during the tasting of samples of liquids at 36.9°C the temperature at the epithelial surfaces of the oral cavity was more uniform than when samples of liquids at 20.4°C were tasted. The former were at almost constant temperature before the taking

Table 10. Statistical treatment of the samples of coefficients of variation, which refer to the experimental conditions of "distilled "water, tap-water, 27°C and 35°C.

$\bar{X}_{C.V.}$ = arithmetical mean, $s^2_{C.V.}$ = variance, and n = number of actual coefficients of variation. The superscripts and subscripts in col. 2 at the given mean values indicate the arithmetical means of the subsamples of coefficients of variation which are obtained under the 27°C and 35°C conditions, respectively. The superscript* at the given t-value in col. 5 means that the hypothesis H_0 is rejected at the 0.05 level. When there is no superscript, H_0 is not rejected.

1	2	3	4	5	6
Sample of coefficients of variation obtained under condition:	$\bar{X}_{C.V.}$	$s^2_{C.V.}$	n	t	Degrees of freedom
"Distilled" water	$14.57^{15.70}_{13.44}$	30.79	72	0.437	142
Tap-water	$14.16^{15.19}_{13.12}$	32.68	72		
27°C	15.45	29.08	72	2.35*	142
35°C	13.28	32.08	72		

(see p. 438). Also the change of temperature at the epithelial surface was small in the course of the tasting, and considerably smaller than when the experiments were carried out with liquids at 20.4°C (Fig. 1, p. 455).

It has already been mentioned that tap-water can be considered to be a more favourable physiological environment for the taste receptors than distilled water. Furthermore, the pH at the receptor surfaces, as can be seen in Fig. 2, is rather more constant during experiments when solutions made up of tap-water are tasted, than when "distilled" water is used as solvent and for rinsing.

If the factors studied, viz. the quality of the water and the temperature, are of importance for the magnitude of the absolute threshold for different substances, it can also be assumed that the standard deviation and coefficient of variation (C.V.) of the thresholds obtained may be influenced to a certain extent by variations in these factors during the experiments.

In testing hypothesis H_0 ("the coefficient of variation is the same under different experimental conditions"), the following procedure was applied. The arithmetical mean ($\bar{X}$) and the variance (s^2) were calculated for the coefficients of variation presented in Table 3 (pp. 444–447) under the experimental conditions of (*a*) "distilled" water (cols. 6 and 10), (*b*) tap-water (cols. 14 and 18), (*c*) 27°C (cols. 6 and 14), and (*d*) 35°C (cols. 10 and 18). However, the coefficients of variation obtained for the compounds 2.5- and 2.6-dinitrophenol and 4-nitroresorcinol were not considered in the calculations because the experiments with these compounds were not completed. The statistics will be found in Table 10, cols. 2 and 3. The mean values of the samples (*a*) and (*b*), like those of the samples (*c*) and (*d*), were then compared with each other with the aid of t-tests.

Table 10 shows that a significant difference between the mean values for the C.V. becomes apparent only on mutual comparison of the statistics for the conditions 27°C and 35°C. The experimental condition 35°C is more favourable than 27°C for

Table 11. Statistical treatment of samples of deviations obtained according to the method described in the text.

The applicable characteristics of the samples studied are marked by crosses in col. 2. The superscript * at the given t-value in col. 5 means that the probability of the hypothesis H_0 (see text) is rejected at the level of 0.05. When there is no superscript, H_0 is not rejected. $\bar{X}_d$ = arithmetical mean, and n = number of variates in the actual sample of deviations.

1	2						3	4	5	6
	Samples of deviations based on data obtained in testing the observer									
	No.:		under condition							
			"dist." water		tap-water					
Line	1	2	27°C	35°C	27°C	35°C	$\bar{X}_d$	n	t	Degrees of freedom
1	×	×	×	×	×	×	−.0096	144	−1.12	143
2	×	×	×		×		−.0184	72	−1.41	71
3	×	×		×		×	−.0008	72	−.075	71
4	×	×	×	×			.0100	72	.837	71
5	×	×			×	×	−.0292	72	−2.44*	71
6	×	×			×		−.0387	36	−2.04	35
7	×	×				×	−.0197	36	−1.33	35
8	×				×	×	−.0282	36	−1.62	35
9		×			×	×	−.0302	36	−1.80	35

obtaining a low standard deviation of the absolute thresholds. The subscripts and superscripts in Table 10, col. 2, show that the effect of temperature is about the same in the two kinds of water.

B. *The variation in time of the absolute threshold*

Goetzl *et al.* (1950) examined the occurrence of diurnal variations in acuity of the sense of taste for sucrose and Irvin and Goetzl (1952) for sodium chloride in normal individuals. Amongst other things, these authors established a more or less continuous fall of the threshold values for these substances in the course of the day until the ingestion of food. For sugar, this drop was, on the average, 30–35 %, and for salt c. 25 % in 3 hours. According to these authors, this seemed to indicate that acuity is in some way linked up with the complex conception of "appetite". Pangborn (1959), however, found no noticeable tendency towards lower thresholds in eight observers under "fasting conditions", when the substances sucrose, sodium chloride, citric acid and caffeine were tested. On the contrary, a trend towards a decrease in sensitivity during a period of increasing appetite was found by Yensen (1959) in studying the fluctuations in taste sensitivity for sucrose, sodium chloride, sulphuric acid and caffeine in two subjects during the course of the day under varying conditions of dietary intake.

Whether or not there is any tendency towards a shifting of the absolute threshold in any direction, when the present data for several substances are combined, has been studied in the following way. The deviation between the logarithms of the

first and fifth threshold values obtained has been calculated for each of the compounds numbered 2–7, 9–16, 19 and 24–26 in Table 1, (p. 436), under each of the four experimental conditions studied and for each observer. Then all the deviations were added up, whereupon the mean, $\bar{X}_d$, and the mean error, m_d, of this sample were calculated. Hypothesis H_0 ("there is no difference in size between the first and fifth threshold") was tested by the application of the t-test $t = (\bar{X}_d - 0)/m_d$. Table 11, line 1, shows that H_0 had to be accepted.

The same hypothesis was applied in the same way to the pairs within subgroups of this sample (Table 11, lines 2–5). These treatments show that there was a trend towards a lowering of the threshold value only during the experiments in which tap-water was used as solvent and for rinsing (line 5). Within sub-groups of the last-mentioned sample, however, a significant difference between the first and fifth threshold did not appear (lines 6–9).

The results obtained seem to be in agreement with those reported by Goetzl *et al.*, Irvin & Goetzl, and Pangborn. Goetzl *et al.* and Irvin & Goetzl, who found a lowering of the threshold with time, used tap-water as solvent and for rinsing, while Yensen and Pangborn, finding that fasting decreased or had no pronounced effect on the sensitivity, made use of distilled water. In the latter case it may be imagined that the tendency towards a fall in the values of the thresholds in question was masked by other factors, antagonistic to the factor of appetite. For example, a gradual rise of the absolute threshold may be expected if the receptors during the tasting of preparations made up with distilled water in the racks intercalated between those with solutions prepared with tap-water become more and more de-activated by the continuous contact with "distilled" water, which, as regards osmotic pressure and content of ions, is very unphysiological in comparison with saliva. A certain rise of the absolute threshold both when "distilled" water and tap-water were used as solvents and for rinsing, is probably furthered by the presence of increasing psychic tiredness in the observers during the rather prolonged testing of each substance, although they did not notice such tiredness.

At the beginning of the experiments the present author considered that a certain risk was involved in the possibility that some of the substances might penetrate into the taste cells and there act as obstacles or activators to metabolic processes of importance for the cells' ability to react to the stimuli. Once the substance has made its way into the cell, its removal may be expected to be much more difficult than when it is found upon or in the cell membrane. This may lead to a gradual falling-off or increase of the sensitivity of the receptors in the course of an experiment. Examples can be found in literature and show that some of the compounds tested affect the rate of enzymatically conditioned reactions. According to Karrer and Viscontini (1947), the activity of cocarboxylase on pyruvate is inhibited by 20 % in a 0.0172 M solution of phloroglucinol at pH 6.2. In a 0.0197 M solution of hydroquinone or resorcinol or in a 0.0231 M solution of phenol the inhibition of the system amounts to 50, 56 and 90 % respectively. Except in the case of hydroquinone the concentrations mentioned are higher than the threshold values of observers Nos. 1 and 2. Furthermore, it is a well-known fact that 2.4-dinitrophenol uncouples the oxydative phosphorylation in concentrations well below the threshold values obtained.

It was pointed out (p. 440) that at the pHs which prevailed during the taste experiments hydroquinone, phloroglucinol, and pyrogallol especially are oxidized in dilute solutions comparatively rapidly. It was therefore necessary to pay special attention to whether there was any remarkable rise or fall with time in the threshold

for any of the substances tested. Scrutiny of Table 2 (p. 443) and of similar tables for the other substances concerned has not, however, revealed any such considerable shifting of the thresholds of any of the compounds studied in the course of the rather prolonged tasting experiments. In principle the method used permits the determination of five thresholds of the same kind in the course of between 50 and 200 minutes.

Furthermore, *t*-tests have been performed with the aim of studying whether or not the threshold values of group II as a whole for different substances changed significantly between the first and second determination. In performing these tests, the difference, d, between the logarithmic value of the thresholds Nos. 1 and 2 of *group II as a whole*, obtained in experiments Nos. A 1–20, B 1–9 and C 1–16 (cf. Tables 1 (p. 436) and 5 (p. 450–451)) has been calculated.

Then the mean value, $\bar{X}_d$, of these 45 differences and its mean error, m_d, have been calculated. However, not all of conceivable differences have been included. Certain differences between threshold values have been excluded, viz. those obtained for resorcinol in the experiments designated "replicate", those between the first and second thresholds represented by the mean value *not* designated "mixed" in experiments Nos. B 1–6, B 8, C 2 and C 5 (cf. Table 5, cols. 5 and 10), those *not* designated "bitter" in experiments Nos. C 2 and C 5 and those *not* involving all the observers in experiment No. A 2). Finally the null hypothesis that there is no difference in magnitude between the first and second threshold, has been tested by the formula $t = (\bar{X}_d - 0)/m_d$. As a *t*-value of 0.020 (44 degrees of freedom) has been obtained, the hypothesis cannot be rejected. The same procedure was also used when considering separately the tests performed under the experimental conditions with pH = 5.1 (experiments Nos. A 1–20 and B 1–9) and pH = 7.8 (experiments Nos. C 1–16). Nor in these cases was it possible to reject the null hypothesis, t being 0.459 (28 degrees of freedom) and 0.742 (15 degrees of freedom) respectively.

A similar procedure was applied to each of the experiments studied above. In these cases the difference, d, between the logarithmic values of thresholds Nos. 1 and 2 of each observer in the group was calculated. After the mean value, $\bar{X}_d$, of the 20 differences and its mean error, m_d, had been estimated, the hypothesis that there is no difference in magnitude between the first and second threshold was tested by the formula $t = (\bar{X}_d - 0)/m_d$ (*t*-quotients not tabulated). However, H_0 could not be rejected in any of the cases. This probably does not mean that the factors affecting the size of the threshold value cancel each other perfectly. Because of the short span of time between the two determinations, the effects of such factors were probably not manifested.

C. *The influence of training upon the level of the threshold*

Since the determinations of thresholds by group II were spread over a longish period and since the majority of the observers (18 individuals) at the beginning of the test period were without previous experience of taste experiments, it appeared to be necessary to study to what extent the magnitude of the mean value of the thresholds in this group was influenced by training. For this reason resorcinol at pH 5.1 was assessed repeatedly (altogether six times) in the course of the test period (see p. 439). Inspection of the results given in Table 5 col. 4 (p. 450) produces the impression of a haphazard variation of the magnitude of the threshold. The lowest and the highest means were obtained from the first and the third experiments, respectively. As will

Table 12. Effect of training upon the magnitude of the threshold for resorcinol.
The *t*-values presented were obtained by the procedure described in the text.

	t-values					Degrees of freedom
Replicate...	1	2	3	4	5	
Experiment No. 1	.892	1.870	.485	1.682	1.145	19
Replicate						
1	—	.812	1.102	.633	.315	19
2		—	1.279	.533	.544	19
3			—	1.397	.584	19
4				—	.199	19

be shown later (p. 491), t-tests were carried out to find out whether there was a significant difference between the threshold values of the observers for a compound at pH 5.1 and those obtained about a month later at pH 7.8. It was therefore also of interest to study whether the differences between the threshold values obtained for resorcinol at pH 5.1 could be considered as accidental or not, according to the *t*-tests. In this operation the arithmetical mean of thresholds 1 and 2 of each observer was first calculated *in each of the actual two experiments compared.* The difference, d, between the logarithms of the two arithmetical means, belonging to the same observer, was then calculated, whereupon the mean value, $\bar{X}_d$, of the 20 differences obtained and its mean error, m_d, was introduced into the formula $t=(\bar{X}_d-0)/m_d$. The *t*-values given in Table 12 show that the hypothesis that there is no difference in magnitude between the thresholds obtained in the two experiments compared could not be rejected in any of the cases. As has been mentioned on p. 448, the interval between two neighbouring concentrations in the series of concentrations offered in the determinations of the threshold was very great. On this account the threshold in question is only roughly estimated for every observer. The training of the observer probably does not result in a noticeable lowering of the threshold. Only when a concentration of a presented substance approximately corresponds to the threshold of recognition, has a trained person an advantage over an untrained one in distinguishing a vague sensation provoked by the substance from other simultaneously experienced but irrelevant perceptions.

D. *The statistical distribution of the thresholds in group II*

The arithmetical mean of the threshold values Nos. 1 and 2 determined for each observer was used as a variate in the calculation of the mean value of the threshold and the standard deviation for each substance and each occasion of testing in group II. The standard deviation was then expressed as a percentage of the actual mean value of the threshold, i.e. as the C.V. The figures which were obtained in this way and which are not reported here give in principle the impression of being of the same order of magnitude, independently of the position in the scale of concentration of the actual mean value of the threshold. These C.V. were in their turn used as variates in the calculation of the mean value $\bar{X}_{\text{C.V.}}$ and the standard deviation $s_{\text{C.V.}}$

Table 13. The statistics which represent the samples of coefficients described in text. $\bar{x}_{C.V.}$ = arithmetical mean, $s_{C.V.}$ = standard deviation, and n = number of actual coefficients of variation.

C.V. of compounds tested, obtained under condition	n	$\bar{x}_{C.V.}$	$s_{C.V.}$
pH 5.1	28	58.9	14.6
pH 7.8	16	59.6	19.6

(Table 13) at pH 5.1 (experiments Nos. A1, A3–20 and B1–9) and 7.8 (experiments Nos. C1–16).

However, not all the conceivable C.V. have been included. Certain C.V. have been excluded, viz. those obtained for resorcinol in the experiments designated "replicate", those for the samples of thresholds, the mean values of which were *not* designated "mixed" in experiments Nos. B1–6, B8, C2 and C5 (cf. Table 5 (p. 450–451), cols. 5 and 10), and those for the samples the mean values of which were *not* designated "bitter" in experiments Nos. C2 and C5. An exceptionally high C.V., viz. 264.2 %, was obtained in the case of phenylthiourea. Two of the observers proved to be "taste blind", i.e. they noticed the substance only in very high concentrations. Their acuity for other substances, however, proved not to differ to a noticeable extent from that of the other members of the groups. Thus there seems to be no correlation between low sensitivity to phenylthiourea and to the other tested substances. The same finding was made by Blakeslee & Salmon as early as 1935, when dealing, amongst other things, with some of the compounds studied here (2.4.6-trinitrophenol, sugar, salt, and vanillin). According to Blakeslee & Salmon (1931), the acuity for phenylthiourea is genetically conditioned. According to Beidler's hypothesis (1961), sensitivity to the compound is dependent on the number of specific receptor sites to which the substance is adsorbed during the process of stimulation.

VI. Taste quality of substances and designation of thresholds obtained

As has been mentioned on p. 434, each of the observers in group I (2 individuals) and group II (20 individuals) supplied in the course of the determinations of thresholds information about the taste quality of the substances, and indicated upon an outline sketch of the tongue the area or areas which had been particularly stimulated. A sketch of this type was then transferred onto transparent celluloid and divided into six zones, according to Fig. 3. In the classical conception, the tip of the tongue is most sensitive to sweet and saline substances, the margins to sour and saline, and the basal part to bitter substances, while the central portion is considered relatively insensitive to taste substances. According to Patton & Ruch (1946), sweet, saline, sour, and bitter substances, in this order, affect the portions of the tongue which have been marked in Fig. 3 with the figures 1, 1 and 2, 2 and 3, and 4. The drawing was superimposed upon each of the outline sketches of the tongue in which the observers in groups I and II had indicated the stimulated areas. In this way the position of the shaded parts with respect to the zones set out in the drawing became

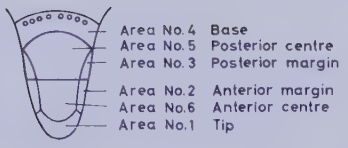
Area No. 4 Base
Area No. 5 Posterior centre
Area No. 3 Posterior margin
Area No. 2 Anterior margin
Area No. 6 Anterior centre
Area No. 1 Tip

Fig. 3. Diagram of the tongue illustrating the areas referred to in the text.

clearly visible. In defining the position of the stimulated receptor surfaces, the following principles have been applied:

1. If 50 % or more of a certain zone was estimated as shaded, the whole of the respective zone of the tongue was counted as stimulated.

2. If the shaded part lay altogether within a certain zone, but was found to be of an extent which did not fulfil the above condition, the corresponding area of the tongue was nevertheless counted as stimulated over its entire extent.

3. A small, shaded part distributed on either side of a boundary separating two zones was referred to the zone containing the greater part. Also in these exceptional cases the entire area in question was counted as stimulated. In Table 14, (p. 476), col. 5*b*, the position of the surface which was especially stimulated by a substance has been given for each of the observers in group I. The taste quality has also been briefly described (col. 6*b*).

As could be expected, concentrations of threshold strength caused diffuse taste sensations. However, the taste quality of the substances as a rule became distinct, when one or two of the strongest concentrations in the series in question were tasted.

In cases in which the solubility of the compounds in water was not a limiting factor, the observers in group I assessed, later in the day, the *taste quality* of solutions of a much higher concentration than any of those previously tasted. This was done with the aim of finding out whether in higher concentrations any new sensations appear or not.

The highest concentration tasted is given in col. 4. On a number of occasions observer No. 1 had the opportunity of tasting some substances at still higher concentrations. In order to save space, the results of these experiments are also reported here. All information in the table which refers to these occasions will be found on those lines, beginning with the superior letter L adjoining the figure 1 in col. 2.

Except in certain particular cases, the observers in group II were offered identical series of concentrations, when the thresholds were determined (cf. p. 449). Column 4 in Table 14 gives the highest concentration tested on *all* observers.

It was found that the observers had different opinions of the taste quality of some of the substances tested. For this reason I have given in col. 6*a* the number of observers in group II who in one and the same tasting observed the taste quality mentioned on the same line in col. 6*b*. In some few cases one or more of the observers were unable to indicate a definite kind of sensation (question mark in col. 6*b*). By inadvertence on my part (I first of all studied the possibility, whether or not a threshold could be determined under the conditions given on p. 438–440), the observers were not offered in these exceptional cases higher concentrations in order to determine the kind of sensation. In the topmost distribution of frequency in col. 5*b* is presented, on each occasion of the testing of group II, the number of observers who in the comprehensive assessment shaded the areas in question upon the tongue.

Table 14. Kind of sensations evoked and area of the tongue stimulated by the compounds studied.

The highest conc. tested and the designations of the threshold values shown in Tables 3 and 5 are presented. Abbreviations: D = "distilled" water, T = tap-water, Sw. = sweet, Bi. = bitter, Sal. = saline, An. = anaesthetizing, As. = astringent, Bu. = burning, t. = taste, and r. = at rinsing. The superscript L in col. 2 refers to experiments performed on a later occasion. The superscripts 1–5 adjoining pH values in col. 3 for resorcinol refer to the different replicates of the threshold estimations performed.

1		2	3	4	5							6			7
					a	b					c	a		c	
a	b				Quality of sensations within areas mentioned in col. 5 b	Number of observers shading area No.					not capable of stating the areas stimulated	Number of observers stating the sensation observed	b Kind of sensation observed	Number of observations of sensation mentioned	In tables 3 and 5 presented thresholds designated
Compound tested															
No.	Name	Group No. Observer No.	pH or kind of solvent used in solutions tested	Highest conc. tested, $\times 10^6$ M		6	1	2	3	4	5				
1	Benzene	I 1	D, T.	5,000								1	None		
		1^L	D.	7,500					1	1	1	1	Bu.(?)		
		2	D, T.	5,000								1	None		
		II	pH 5.1	5,000									None	33	
													Metal(?)	1	
													An.(?)	1	
													Sour(?)	1	
													(?)	4	
2	Nitrobenzene	I 1	D, T.	3,580			1					1	Sw.		Sw.
		2	D, T.	3,580			1					1	Sw.		Sw.
		II	pH 5.1	800		1	17	8	2	1		20	Sw.		Sw.
			pH 7.8	1,600		1	19	8	2			20	Sw.		Sw.
3	1.2-Dinitrobenzene	I 1	D, T.	1,000			1					1	Sw.		Sw.
		2	D, T.	1,000			1					1	Sw.		Sw.
		II	pH 5.1	400		4	15	8	3			20	Sw.		Sw.
			pH 7.8	800		4	19	6	1			20	Sw.		Sw.
4	1.3-Dinitrobenzene	I 1	D, T.	1,000						1		1	Bi.		Bi.
		1^L	D.	1,550					1	1		1	Bi.		
		2	D, T.	1,000						1		1	Bi.		Bi.
		II	pH 5.1	400		1	2	4	5	17	1	20	Bi.		Bi.
			pH 7.8	800		1	1	4	9	17	2	20	Bi.		Bi.
5	1.4-Dinitrobenzene	I 1	D, T.	500						1		1	Bi.		Bi.
		2	D, T.	500						1		1	Bi.		Bi.
		II	pH 5.1	200		1	2	3	5	13	1	20	Bi.		Bi.
			pH 7.8	400		2	2	3	10	14		20	Bi.		Bi.
6	1.3.5-Trinitrobenzene	I 1	D, T.	500						1		1	Bi.		Bi.
		2	D, T.	500						1		1	Bi.		Bi.
		II	pH 5.1	80			2	2	8	17	1	20	Bi.		Bi.
			pH 7.8	160				2	6	17	3	20	Bi.		Bi.

7 Phenol	I	1	D, T.	100,000			1							1	Sw., An.		Sw.
		2	D, T.	100,000			1							1	Sw., An.		Sw.
	II		pH 5.1	16,000		2	19	5	5	4	1			8	Sw.		Sw.
					Total Sw.	2	17	5	5	4	1			8	Sw., An.		
					Only Sw.		8	1	2	2				2	Sw., Bu.		
					Total An.	1	9	3	1	1				1	Sw., An., Bi.		
					Only An.		2							1	Sw., Bi.		
					Total Bu.		2	1	2	1							
					Total Bi.	2	1	1			1						
	II		pH 7.8	16,000		4	19	6	2					6	Sw.		Sw.
					Total Sw.	4	17	6	2					11	Sw., An.		
					Only Sw.		5	3	2					1	Sw., Bu.		
					Total An.	4	12	3						1	Sw., Bu., An.		
					Only An.		1							1	(?)		
					Total Bu.		2										
8 Pyrocatechol	I	1	D.	100,000			1							1	An., Bu.		Anæsthesia
		2	D.	100,000			1							1	An., Bu.		Anæsthesia
	II		pH 5.1	100,000											An.	13	
															An., Bu.	13	
															An., Bi.	5	
															An., Bu., Bi.	3	
															Bu.	3	
															Bu., Bi.	2	
															Bi.	1	
9 Resorcinol	I	1	D, T.	50,000			1							1	Sw.		Sw.
		1[L]	D.	67,800			1							1	Sw.		
		2	D, T.	50,000			1							1	Sw.		Sw.
	II		pH 5.1	16,000		1	18						2	20	Sw.		Sw.
			pH 5.1[1]	16,000		1	17	6	3	3			1	17	Sw.		Sw.
					Total Sw.	1	17	6	3	3			1	3	Sw., Bi.		
					Only Sw.	1	16	4	2	2							
					Total Bi.		1	2	1	1							
			pH 5.1[2]	16,000		3	13	4	4	1			3	19	Sw.		Sw.
					Total Sw.	3	13	4	4	1			3	1	Sw., An.		
					Only Sw.	3	12	4	4	1			3				
					Total An.		1										
			pH 5.1[3]	16,000		1	19	3	3	4	1			16	Sw.		Sw.
					Total Sw.	1	19	3	3	3	1			2	Sw., Bi.		
					Only Sw.	1	18	2						1	Sw., Bu.		
					Total Bi.		1	1	1	2				1	Sw., Metal		
					Only Bi.					1							
					Total Bu.				1	1							
					Total Metal					1	1						
			pH 5.1[4]	16,000		1	17	8	3	1				19	Sw.		Sw.
					Total Sw.	1	17	8	3	1				1	Sw., Bi.		
					Only Sw.	1	16	7	2								
					Total Bi.		1	1	1	1							

Table 14 (*continued*)

1	2	3	4	5							6	7
				6	1	2	3	4	5			
		pH 5.1[5]	16,000		3	17	4	2	4		17 Sw.	Mixed sensations, $n = 20$
				Total Sw.	3	16	4	1	2		1 Sw., Bi.	
				Only Sw.	3	15	3	1	2		2 Bi.	Sw., $n = 18$
				Total Bi.		2	1	1	2			
				Only Bi.		1		1	2			
		pH 7.8	16,000		1	19	8	4	1	1	18 Sw.	Sw.
				Only Sw.	1	17	7	4	1	1	2 Sw., Bi.	
				Total Bi.		2	1		1			
10 Hydroquinone	I 1	D, T.	30,000			1					1 Sw., As.	Sw.
							1	1			1 Sw., Sal.	
	1[L]	D.	500,000			1	1	1			1 First Sw., then Metal	
	2	D, T.	30,000			1					1 Sw., As.	Sw.
							1	1			1 Sw., Sal.	
	II	pH 5.1	80,000		3	16	9	5	3		12 Sw.	Sw.
				Total Sw.	3	16	9	5	3		6 Sw., Bi.	
				Only Sw.	3	11	5	3	1		2 Sw., Bu.	
				Total Bi.		3	4	2	2			
				Total Bu.		2						
		pH 7.8	80,000		5	11	4	3	2	3	6 8 Sw.	Mixed sensations, $n = 20$
				Total Sw.	4	11	4	3	1	3	3 5 Sw., Bi.	
				Only Sw.	3	8	2	2	1	2	1 1 Sw., Bi., Sal.	Sw., $n = 19$
				Total Bi.	2	2	2	1	1	1	2 2 Sw., Sal.	
				Only Bi.	1				1		1 Bi.	
											3 (?)	
11 Pyrogallol	I 1	D, T.	1,000,000				1				1 Bi., As.	Bi.
					1	1	1				1 Sw. t. r.	
	2	D, T.	1,000,000				1				1 Bi.	Bi.
					1	1	1				1 Sw. t. r.	
	II	pH 5.1	80,000		6	18	8	14	7	8	2 20 Bi.	Bi.
											20 Sw. t. r.	Sw. t. r.
				Total Bi.	3	4	8	14	7	8	2	
				Only Bi.	2		5	12	7	7		
				Total Sw. t. r.	4	18	3	2		1	2	
				Only Sw. t. r.	3	14						
		pH 7.8	80,000		3	18	7	14	11	8	2 20 Bi.	Bi.
											20 Sw. t. r.	Sw. t. r.
				Total Bi.	3		7	14	11	7	1	
				Only Bi.	3		7	14	11	7	1	
				Total Sw. t. r.		18				1	2	
				Only Sw. t. r.		18				1	2	

12	Phloroglucinol	I	1	D, T.	10,000			1								1	Sw.	Sw.
			1^L	D.	30,000			1								1	Sw.	
			2	D, T.	10,000			1								1	Sw.	Sw.
		II		pH 5.1	4,000		2	19	5	3	1	1			20	Sw.	Sw.
				pH 7.8	4,000		2	16	9	3	1	1			20	Sw.	Sw.
13	*o*-Nitrophenol	I	1	D, T.	4,000			1								1	Sw.	Sw.
			1^L	D.	18,900			1								1	Sw.	
											1					1	Bi.	
			2	D, T.	4,000			1								1	Sw.	Sw.
		II		pH 5.1	8,000		4	15	6		2			2	18	Sw.	Sw.
						Only Bi.					1				1	Sw., Bi.	
															1	(?)	
				pH 7.8	4,000		3	15	10	4	3				18	Sw.	Sw.
						Only Bi.					2				2	Sw., Bi.	
14	*m*-Nitrophenol	I	1	D, T.	4,000			1								1	Sw.	Sw.
			2	D, T.	4,000			1								1	Sw.	Sw.
		II		pH 5.1	16,000		1	16	8	2				1	20	Sw.	Sw.
				pH 7.8	16,000		2	16	8	2	1			1	20	Sw.	Sw.
15	*p*-Nitrophenol	I	1	D, T.	5,000			1								1	Sw.	Sw.
			1^L	D.	41,000			1								1	Sw.	
							1	1	1							1	Bu.	
			2	D, T.	5,000			1								1	Sw., As.	Sw.
		II		pH 5.1	16,000		4	17	10	3	1			2	19	Sw.	Sw.
						Total Bu.			1						1	Sw., Bu.	
				pH 7.8	8,000		4	15	5	3	1	1			19	Sw.	Sw.
						Total An.		1	1		1				1	Sw., An.	
16	2.4-Dinitrophenol	I	1	D, T.	5,000			1			1				1	Sw., Bi.	Sw. and Bi.
			1^L	D.	8,000			1			1				1	Sw., Bi.	
							1	1	1						1	Bu., "Etching"	
			2	D, T.	5,000			1			1				1	Sw., Bi.	Sw. and Bi.
		II		pH 5.1	4,000		1	17	5	5	15	1		1	13	Sw., Bi.	Sw.
						Total Sw.	1	17	5	2	7	1			5	Sw.	
						Only Sw.	1	13	2	1	1				1	Sw., Bu.	
						Total Bi.		3	2	3	13	1			1	(?)	
						Only Bi.				3	8						
						Total Bu.		1	1	1	1						
				pH 7.8	4,000		1	19	5	6	13			1	15	Sw., Bi.	Sw.
						Total Sw.	1	19	5	4	5			1	5	Sw.	
						Only Sw.		12	1	1				1			
						Total Bi.	1	7	4	5	13						
						Only Bi.				2	8						
17	2.5-Dinitrophenol	I	1	D, T.	5,000			1								1	Sw.	Sw.
							1	1	1						1	Bu.	
			2	D, T.	5,000			1								1	Sw.	Sw.
								1	1	1						1	Bu.	

Table 14 (*continued*)

1	2	3	4	5							6			7
					6	1	2	3	4	5				
	II	pH 5.1	10,000			2	14	6	6	3	1	5	9	Sw.		Sw.
				Total Sw.		1	13	5	4	2		4	7	Sw., Bu.		
				Only Sw.		1	5	2	1			3	3	Sw., Sal.		
				Total Bu.			4		2	1		1	1	(?)		
				Only Bu.					1							
				Total Sal.			3	3	2	1						
	II	pH 7.8	10,000			3	19	14	9	6			14	Sw., Bu.		Mixed sensations, $n = 20$
				Total Sw.		2	18	10	2	2			2	Sw., Bu., An.		
				Only Sw.		1	8	2					1	Sw., Bu., Sal.		Sw., $n = 18$
				Total Bu.		2	11	12	8	5			1	Sw., Bu., Bi.		
				Only Bu.		1	1	3	5	3			1	Bu., Sal.		
				Total Sal.			1	1	1	1			1	Bu.		
				Only Sal.					1	1						
				Total Bi.			1		1	1						
				Total An.			1	2	1	1						
18 2.6-Dinitrophenol	I 1	D, T.	5,000			1	1	1			1		1	(?)		Mixed sens.
	1^L	D.	8,380			1					1		1	As.		
	2	D, T.	5,000			1	1	1	(?)	(?)	1		1	(?)		Mixed sens.
	II	pH 5.1	5,000											None	19	
														Sal.	4	
														Sw.	3	
														Sw., Bi.	1	
														Bi.	2	
														Sour	2	
														An.	2	
														'Stale"	1	
														(?)	6	
19 2.4.6-Trinitrophenol	I 1	D, T.	50						1				1	Bi.		Bi.
	1^L	D.	100						1				1	Bi.		
	2	D, T.	50						1				1	Bi.		Bi.
	II	pH 5.1	8			1		2	4	20			20	Bi.		Bi.
		pH 7.8	8				3	5	4	18		1	16	Bi.		Bi.
				Total Bi.			2	4	4	16		1	1	Bi., Sw.		
				Only Bi.			1	2	3	15		1	1	Bi., Sal.		
				Only Sw.			1	1					1	Bi., Bu.		
				Total Sal.			1	1		1			1	(?)		
				Total Bu.				1	1							
20 4-nitroresorcinol	I 1	D, T.	10,000				1						1	Sw.		Sw.
	2	D, T.	10,000				1						1	Sw.		Sw.
21 Benzaldehyde	I 1	D, T.	20,000				1						1	None, painful.		
	2	D, T.	20,000				1						1	None, painful		

| | | |

Table 14 (*continued*)

1	2	3	4	5							6		7
				6	1	2	3	4	5				
29 Potassium nitrite	II	pH 5.1	12,800		5	16	10	6	5	1	1	11	Sw.	Mixed sensations, $n = 20$
				Total Sw.	5	16	8	4	4	1		3	Sw., Sal.	Sw., $n = 18$
				Only Sw.	3	10	4	1	2	1		2	Sw., Bi.	
				Total Sal.	2	3	4	3	2			2	Sal.	
				Only Sal.			2	2	1			1	(?)	
				Total Metal		1						1	Sw., Metal	
				Total Bi.		2	2	2	1					
30 Nitromethane	II	pH 5.1	800,000		6	16	9	3	7	1	3	8	Sw., Bi.	Sw.
				Total Sw.	6	16	9	3	5	1	2	5	Sw.	
				Only Sw.	1	5	2				2	2	Sw., Bi., Bu.	
				Total Bi	2	6	6	2	6	1	1	2	Sw., Sour	
				Only Bi.					2		1	2	Sw., Bu.	
				Total Sour	2	1	1	1	1			1	Sw., Cool	
				Total Bu.		4	1							
				Total Cool.	1	1								
31 Nitroethane	II	pH 5.1	320,000		5	18	5	3	4	2	1	14	Sw.	Sw.
				Total Sw.	5	18	5	3	1	2	1	3	Sw., Bi.	
				Only Sw.	3	15	2	1		1	1	1	Sw., Bi., Bu.	
				Total Bi.	1		1	2	3	1		2	Sw., Bu.	
				Only Bi.					2					
				Total Bu.	1	3	2							
32 1-Nitropropane	II	pH 5.1	90,000		3	15	6	4	9	1	3	12	Sw., Bi.	Mixed sensations, $n = 20$
				Total Sw.	2	15	6	3	5	1	3	4	Sw.	Sw., $n = 20$
				Only Sw.		8	3				1	4	Sw., Bu.	
				Total Bi.	2	5	3	4	7	1	2			
				Only Bi.				1	3					
				Total Bu.	1	2			2					
				Only Bu.	1				1					
33 2-Nitropropane	II	pH 5.1	72,000		2	8	12	9	9	1	2	8	Bi.	Mixed sensations, $n = 20$
				Total Sw.		3	2	1	1			2	Sw.	Bi., $n = 14$
				Only Sw.		3	2	1	1			1	Bi., Sour	
				Total Bi.	2	2	6	6	6	1		1	Bi., Sw., Bu., Sour	
				Only Bi.	1	1	3	5	5	1		1	Bi., Cool., An.	
				Total Bu.	1	2	3		1			2	Bu.	
				Only Bu.		2	1		1			1	Bu., Cool	
				Total An.		1	2	1				3	(?)	
				Only An.			1	1						
				Total Sour		1	2							
				Total Cool.	1		2	1	1					

34	Methanol	II	pH 5.1	10,000,000		4	15	7	4	15	2	3	20	Sw., Bi.	Mixed sensations, $n = 20$
					Total Sw.	4	15	7	4	10	2	2			Bi., $n = 20$
					Only Sw.	1	5	3							Sw., $n = 20$
					Total Bi.	3	10	4	4	15	2	3			
					Only Bi.					5		1			
35	Ethanol	II	pH 5.1	10,000,000		4	15	5	8	14	2	3	16	Sw., Bi.	Mixed sensations, $n = 20$
					Total Sw.	4	14	5	6	7	2	3	4	Sw., Bi., Bu.	Bi., $n = 20$
					Only Sw.	2	8	2							Sw., $n = 20$
					Total Bi.	2	6	3	8	14	2	3			
					Only Bi.				2	7					
					Total Bu.	2	2	1	2	1					
					Only Bu.		1								
36	2-Propanol	II	pH 5.1	4,000,000		5	15	5	4	15	1	2	13	Sw., Bi.	Mixed sensations, $n = 20$
					Total Sw.	3	13	2	1	8		1	2	Bi.	Bi., $n = 19$
					Only Sw.		4						1	Bi., Sal.	Sw., $n = 14$
					Total Bi.	5	9	5	4	14	1	1	1	Bi., Sour	
					Only Bi.	2	1	3	2	4	1		1	Bi., Bu.	
					Total Bu.		1			1			1	Sw., Bu.	
					Total Sour.				1	1			1	(?)	
					Total Sal.					1					

Some of the substances were found capable of simultaneously producing more than one taste quality or kind of sensation. The observers in group I often found these different qualities localized in different parts of the tongue. As could be expected, the observers in group II also shaded in such cases approximately similar parts of the outline sketch. It was, however, surprising to see how often they found it difficult to connect the shaded portions with the different qualities. They were asked to refrain from guesses, and they were not in these cases given a new series for tasting, since unfortunately a new test would have taken too much time.

In the distribution of the frequency, which follows in col. 5*b* for each substance and each occasion of testing of group II after the first distribution, an account is given in the same way of the distribution of the shaded areas upon the tongue for each of the taste qualities mentioned by the observers. Upon a line headed "total" in col. 5*a*, followed by the indication of a taste quality, col. 5*b* gives the number of cases in which the pertinent areas have been shaded and in which the taste quality in question, along with other sensations, might have been perceived in these spots. If, on the other hand, the line is headed "only", followed by the taste quality, the distribution applies to the number of persons who simultaneously with the tasting observed and noted the actual taste quality in the spots.

The appearance of the distributions is dependent on the ability of the observers to indicate precisely the position of the stimulated area of the tongue and to transfer their subjective impressions to the outline sketch in question and also on the way in which I have interpreted this outline sketch. On account of the fact that for the assessment the observers had as a rule been offered identical series of concentrations, while their thresholds of recognition lay at different levels, their comprehensive assessments are based upon an individually different number of observations (min. 2, max. 10) of the taste quality and of the localization of the stimulated surface of the tongue. It is to be expected that observers with thresholds of recognition immediately below the highest of the tested concentrations will supply less precise and also less reliable information about their experiences than their more sensitive companions. Also in the latter there is certainly a tendency to shade greater areas upon the outline sketch of the tongue under the influence of the taste sensations obtained during the tasting of the highest concentration, and this in spite of the fact that they had been told to shade only those areas of the tongue which had been stimulated in the neighbourhood of the threshold of recognition.

From the above it becomes evident that the data collected for each substance about the location of the principally stimulated tongue areas are only approximate. The same remark applies to the distributions of frequency obtained. More detailed and precise information could without doubt have been obtained, if every observer had been instructed to indicate the stimulated parts of the oral cavity in a special sketch immediately after each assessment. Under these conditions it would have been possible to report the distribution of frequency within the group for different levels with absolute thresholds of the different observers as a starting-point. However, such an investigation was out of the question, since it would have taken too much time, and my principal aim on this occasion was to determine the thresholds for the substances in question.

Before the start of the experiments the members of group II were told to enter in the journal after the assessment of every single sample the first letter of the word or words describing the sensory perceptions observed (see p. 438). According to the observers themselves, they were able to carry out this task without being pressed

for time. They considered it as helpful in giving a comprehensive account of the taste quality. It was my intention to make use of these observers' notes in an attempt to establish whether or not the absolute threshold for each observer was bound up with the same quality of taste.

If one and the same taste quality is found in all the reports given by the observers, the mean threshold value of the group is made to represent this one; if not, the mean value is mixed. In the latter case also the threshold was calculated for the taste quality which either alone or in combination with other tastes was reported by the majority of the observers. Those who did not state the taste quality are also included in this group, on the assumption that, if they had been permitted to taste higher concentrations, they would have reported the most frequently noticed one in the judgements. The thresholds calculated and designated in this way are presented in Table 14, col. 7, and in Table 5 (p. 450–451) cols. 4 and 9.

Commentary on the results of the taste experiments given in Table 14

Benzene

Group I. In the concentration 5000×10^{-6} M the substance was tasteless. In three cases out of six the concentration 7500×10^{-6} M produced a very vague burning sensation upon the indicated areas of the tongue.

Group II. The observers tasted the given concentration, 5000×10^{-6} M (pH 5.1), twice in the course of one tasting, when at the same time solutions of other compounds which were also suspected of being without sweet and bitter taste were inserted in random order into the series in question (see p. 438). Thus $2 \times 20 = 40$ assessments of the taste of benzene were obtained from group II. In these cases I considered it simplest to report only the frequency of assessments of the same kind (col. 6*c*). As can be seen, different reports about the quality of the taste were given by the observers who considered that the substance possessed a taste. In these cases the assessment of the taste quality is probably based upon guesses.

Phenol

Group I. Ten to twenty seconds after taking a concentration of the compound above the threshold, the observers noticed upon the tip of the tongue a feeble, prickling sensation. The epithelium felt slightly stiff and on this account the substance was assumed to act as a local anaesthetic. With regard to the time of reaction, intensity, duration, and extension upon the tongue, the sensation is dependent on the concentration. In high concentration the anaesthetic effect is very pronounced.

Group II. The assessments agree well with those obtained by group I. Sweet taste was noticed by all persons. The action of the substance as a local anaesthetic is localized to the tip of the tongue and is more pronounced at the higher pH. Altogether 6 out of 20 persons noticed a burning or bitter component in the taste at one of the two tastings. As can be judged from the diagram, these sensations are restricted to the tip of the tongue. I consider that they probably represent the vague, prickling sensation experienced by the observers in group I.

Pyrocatechol

Group I. The effect of the substance as a local anaesthetic appears in low concentrations first upon the tip of the tongue. The sensation resembles that produced by phenol, and likewise depends on the concentration, with regard to the time of reac-

tion, intensity, duration and extension upon the tongue. The given threshold represents the concentration at which an effect of local anaesthesia is produced within 6 seconds after the taking. Solutions of about double that strength produce a burning sensation upon the tip and the back of the tongue. In the highest of the tasted concentrations the effect of local anaesthesia was very obvious. The insensibility of the epithelium of the tongue lasted for several minutes. A vague, burning sensation was observed all over the tongue.

Since I was interested mainly in studying the effect of the temperature and quality of the water upon the level of the absolute thresholds for sweet and bitter substances, the plan of the experiments with pyrocatechol was altered so as to comprise the determination of only one threshold for each of the conditions in question.

Group II. The same applies to the experiments with group II. The observers tasted the offered concentration ($100{,}000 \cdot 10^{-6}$ M) twice. The impressions of the substance obtained by group I here received additional confirmation. Pyrocatechol acts as local anaesthetic and produces a burning sensation upon the tongue. A bitter component of taste was observed in altogether 11 cases. This sensation might have been mistaken for the burning one.

Resorcinol

Group I. Both observers found the substance purely sweet, even in higher concentrations. Stimuli were found to act mainly upon area I.

Group II. Within the interval of concentrations examined the sweet taste of the substance predominates. At the altogether seven tastings, however, one or another of the observers now and then reported experiencing other sensations. This appears from the survey given below of the kinds and number of sensations reported by the different observers in group II.

The number of reports on the sensation given by each of the actual observers in col. 1.

Number of observers	Sweet	Bitter	Sweet and bitter	Sweet and anaesthetic	Sweet and burning	Sweet and metallic
2	5		2			
3	6		1			
1	5		1	1		
1	5		1		1	
1	5	1				1
1	6	1				
11	7					

Obviously the observers in question had not repeated their assessments frequently. Against the background of the varying assessment of taste quality by the observers, the threshold determined in replicate 5 becomes unspecified, i.e. mixed. In this case the mean threshold value of the 18 observers who reported the sweet taste of the compound has also been calculated.

Hydroquinone

Group I. At surfaces 2 and 3 a diffuse mixed taste was observed, like that obtained, for example, with a mixture of sugar and salt in suitable proportions. Furthermore

the anterior part of the tongue felt dry and harsh. The observers interpreted the experience as indicative of an astringent effect of the substance upon the epithelium.

According to observer No. 1, the sweet taste in the higher concentration was of short duration. On its disappearance a metallic sensation made itself felt along the margins of the tongue.

Group II. Side by side with the sweet taste, the bitter taste was noted in a surprisingly large number of cases. Four out of 20 observers described the quality of the taste as sweet and bitter at both of the tastings. The other carried out the assessment only once.

As will be seen from the table, the mean threshold value obtained for the group at the higher pH is a mixed one. In calculating the threshold representing the sweet taste, the data for the observer who reported a bitter taste have been excluded.

Pyrogallol

Group I. The two observers were of the opinion that the sensation caused by the substance was very reminiscent of bitter taste. Observer No. 1 at the same time considered it somewhat astringent. Both persons localized the taste sensation in area 2. A solution of 1 M also stimulated area 6 and the inside of the cheeks.

When after each assessment the mouth was rinsed with water, sweet taste was perceived in areas 1, 2, and 6. The threshold was found to lie below the lowest concentration of the series. No special experiments were performed in order to determine its level.

Group II. All observers designated the taste quality as bitter. As can be seen from Table 14, it was localized especially in area 3, but also to a large extent in the adjoining areas 4, 5, and 2. The distribution thus differs essentially from that found on tasting the other bitter substances. The substance seems to have stimulated receptors for bitter taste which have another distribution than those activated by the other bitter compounds tested.

In contrast to the observers in group I, those in group II found sweet taste occurring in connection with the rinsings strictly localized to the tip of the tongue.

Since the series presented covered a much wider span of concentrations than those tasted by group I, it was possible to determine at one and the same tasting both the threshold for bitter taste and that for sweet after-taste at the first rinsing.

o-Nitrophenol

Groups I and II. Only two of the 20 observers in group II noticed bitter taste at the posterior part of the tongue, like observer No. 1 in group I, on tasting the substance in higher concentrations.

p-Nitrophenol

Groups I and II. One and the same observer in group II observed a burning component in the taste of solutions at the lower pH level tested and a sensation which was interpreted as anaesthesia at the higher one. In group I, observer No. 2 reported a supplementary astringent sensation. A considerably higher concentration caused in observer No. 1 a burning sensation in addition to sweet taste.

2.4-*Dinitrophenol*

Group I. Already in the neighbourhood of the absolute threshold the substance caused sweet and bitter taste which was localized mainly in areas 1 and 4. Observer No. 2 noticed taste sensations also upon the inner side of the cheeks. According to

observer No. 1, an intensely burning, "etching" sensation was predominant at the concentration $8000 \cdot 10^{-6}$ M. In his opinion the thresholds for sweet and bitter taste coincide.

Group II. Since the bitter taste did occur with a lower frequency than the sweet one, the thresholds have to be considered as representing the latter.

2.5-*Dinitrophenol*

Groups I and II. Side by side with sweet taste, a burning sensation was in most cases reported by the observers in group II. The frequency of such assessments increases considerably, if the pH of the solutions is raised by about one unit. The burning component was observed also by the observers in group I. Of the other observed taste components, the saline one was noticed on both occasions of tasting by only one person. At the higher pH level two subjects did not report a sweet component among the sensations evoked by the compound. Mean threshold values representing both a mixed and a sweet taste have therefore been calculated.

2.6-*Dinitrophenol*

Group I. The observers were unable to distinguish the qualities entering into the mixed taste. After the introduction into the mouth of the concentration $8380 \cdot 10^{-6}$ M the tongue felt somewhat dry and harsh. This may indicate that "cutaneous" sensations enter into the mixed taste. Thus the determined threshold could not be more definitely specified.

Group II. About half of the members of the group considered the substance as tasteless. Nine observers noticed a taste in the solutions, but opinions about the quality of the taste were widely divergent, as can be seen from the table.

Benzaldehyde

Groups I and II. The observers in group I considered the substance as tasteless. After having been retained in the mouth for a few seconds, however, it caused pain upon the tip of the tongue. Most of the observers in group II also considered the substance as productive of pain. Two persons were of the opinion that both solutions acted as local anaesthetics.

Vanillin

Group I. The substance caused painful sensations, especially upon the under side of the tip of the tongue. On this occasion the solution was $100.000 \cdot 10^{-6}$ M (supersaturated). Observer No. 1 tasted a single series (pH = 5.1) and found that the threshold for burning sensation was situated at about $23{,}000 \cdot 10^{-6}$ M.

Phenylthiourea

Group II. Two among the 20 observers in group II observed the substance only when tasting very high concentrations. These so-called non-tasters were of the opinion that at high concentrations the taste had both a sweet and a saline component. In this particular case the thresholds were calculated separately for the 18 normal tasters and for the two non-tasters.

Sodium nitrite

For this substance all observers with one exception noted the sweet taste. On this account the thresholds for all 20 persons and that for the 19 who recorded sweet taste are presented in Table 5 (p. 451), col. 4. The two observers forming group I

described the sensation adjoining the sweet taste as metallic. A similar electrolyte taste is experienced on tasting preserved foods which have been kept too long in open tins.

Potassium nitrite

The opinions agree closely with those reported for sodium nitrite. Observer No. 1 considered it as exclusively sweet, while observer No. 2 identified the taste with that of sodium nitrite.

Nitromethane

Half of the participants, i.e. 10 persons, observed a bitter component in the taste. However, all observers noticed sweet taste at lower or in exceptional cases at the same concentrations as additional sensations. These exceptional cases were 3 in number, among them the two observers in group I.

Nitroethane

Sweet taste was always observed at the same or at lower concentrations as the bitter one. The observers forming group I considered the substance as sweet and bitter, and localized the bitter taste in area 4.

1-*Nitropropane*

Five observers out of 20 considered bitter taste as predominant, since they observed it at considerably lower concentrations than sweet taste. The calculated mean value of the threshold representing sweet taste can thus be considered as a combination of absolute thresholds and thresholds of recognition for the substance. The author and Mrs. Öhman-Saks reported sweet taste in area 1 and bitter taste in areas 4 and 3–4, respectively.

2-*Nitropropane*

In agreement with the other, structurally closely related substances tested, 2-nitropropane also seems capable of causing a burning sensation. It should be noted that the mean threshold value representing bitter taste, has been calculated on data obtained for the 11 observers reporting bitter taste and the three subjects who were unable to state the taste quality.

Methanol

Since all observers had reported sweet and bitter taste, the level of the absolute threshold for the group and of the thresholds representing sweet and bitter taste were calculated. Bitter taste became obvious to most persons at concentrations which were lower than those producing sweet taste (cf. the level of the thresholds).

Ethanol and 2-propanol

The distribution of frequencies agree closely with that obtained for methanol. A burning component has been noticed.

Summary

According to the assessments carried out by the observers in group I and II, the substances nitrobenzene (substance No. 2 according to Table 14, col. 1), 1.2-dinitrobenzene (3), phenol (7), resorcinol (9), hydroquinone (10), phloroglucinol (12),

o-nitrophenol (13), *m*-nitrophenol (14), *p*-nitrophenol (15), 2-4-dinitrophenol (16), 2.5-dinitrophenol (17), 4-nitroresorcinol (20), sucrose (26), sodium nitrite (28), potassium nitrite (29), nitro methane (30), nitro ethane (31) and 1-nitropropane (32) mainly stimulated areas 1 and 2. The differences in the distribution of frequencies are certainly expressions of a haphazard variation (cf., for example, the distribution of frequencies representing resorcinol, substance No. 9).

It is, however, obvious that several of the substances mentioned are capable of influencing other excitable systems than those for sweet taste. This applies particularly to the compounds 2.4-dinitrophenol (16), nitromethane (30), and 1-nitropropane (32), for which several observers in group II reported a bitter component in the taste in the neighbourhood of the absolute threshold but also to *o*-nitrophenol (13) according to observer No. 1 and to nitroethane (31) according to observers in group I. They form a more or less marked transition to the substances methanol (34) and ethanol (35), and perhaps also 2-propanol (36), which, as can be judged from the distributions of frequencies, have a strong stimulating effect also upon area 4, and which can be designated as sweet-bitter, both adjectives carrying the same weight. Furthermore phenol (7) seems to have an anaesthetic effect, hydroquinone (10) produces a mixed taste, which was described by several observers in group II as sweet and bitter, and 2.5-dinitrophenol (17) stimulates receptors of pain, while sodium nitrite (28) and potassium nitrite (29) cause sweet taste with a saline or metallic component.

For bitter taste, we may refer mainly to the substances 1.3-dinitrobenzene (4), 1.4-dinitrobenzene (5), 1.3.5-trinitrobenzene (6), 2.4.6-trinitrophenol (19), quinine hydrochloride (27) and phenylthiourea (25). As can be judged from the diagrams, they stimulate especially area 4. Pyrogallol (11) seems to have its strongest effect upon area 3. For this reason, it may be imagined that the substance acts upon receptors which on stimulation give the impression of bitter taste, but whose distribution differs somewhat from that of the receptors excited by the other bitter substances. The substance in question also proved to cause sweet taste on removal from the receptor surfaces. According to the reports of the observers in group II, the distribution of the receptor surfaces which had been stimulated on these occasions was roughly the same as that stimulated by the application of sweet substances. 2-Nitropropane (33) proved to cause preponderantly bitter taste within the group, but a large proportion of the observers gave a different opinion about the taste quality. Finally, sodium chloride (27) affected mainly areas 1 and 2, as could be expected.

The taste quality of a large number of the substances I tested was included in Cohn's paper (1914). The results I obtained do not agree with the information given by Cohn for several substances. According to him, phenol is sweet and slightly irritating, pyrocatechol is bitter, benzaldehyde causes "etching", vanillin is hot and both methanol and ethanol are tasteless and burn the tongue.

The reports of the taste quality of ethanol which are given in this investigation agree better with the results obtained by Diamant *et al.* (1963) in experiments made on 13 subjects. Bitter, burning and sweet were the most frequently reported sensations within this group, bitter being observed by 13 subjects, burning by 12 and sweet by 7. The differences observed between the data presented by Diamant *et al.* and by the present author may possibly be attributed to differences in the methods applied.

VII. Statistical treatment of the theshold values

A. *Group II as a whole*

As has been mentioned above (pp. 438–439), the thresholds of the 20 observers were determined for a number of substances in "distilled" water at two different pHs. The *t*-test was applied in order to determine whether or not there was a noticeable difference in value between the thresholds obtained for an actual compound under these two experimental conditions. For each observer in the group the difference, d, was formed between the logarithms of the arithmetical means of thresholds No. 1 and No. 2 at pH 5.1 and 7.8, respectively, whereupon the quotient $t = (\bar{X}_d - 0)/m_d$ was calculated. It has to be pointed out that the lapse of time between the tastings carried out with solutions at pH 5.1 (experiments Nos. A1, A3–14, A17, A19 and A20) and at 7.8 (experiments Nos. C1–16), respectively, was about one month for each substance (cf. Tables 1 (p. 436) and 5 (p. 450)). As has been shown earlier (pp. 472–473), the threshold for resorcinol at pH 5.1 did not change appreciably during the whole test period. Thus we have reason to assume that the greater difference which is found for some compounds between the two mean threshold values of observers in group II may be a consequence of the difference in the pHs of the solutions on the two occasions considered.

Results

The results of the *t*-tests are presented in Table 5, col. 12. Two asterisks (**) and one asterisk (*) in connection with the *t*-values presented mean that the hypothesis H_0 ("there is no difference in magnitude between the thresholds obtained at pH 5.1 and 7.8, respectively") is rejected at the level of 0.01 and 0.05 respectively. No asterisk indicates that H_0 could not be rejected. In view of the fact that several *t*-tests have been performed, there is reason to suspect that one or two of the *t*-values have attained significant magnitude merely by chance.

A significant difference between the two mean threshold values compared is apparent for only five of the 16 substances examined. This applies to 1.2-, 1.3- and 1.4-dinitrobenzene, *p*-nitrophenol and 2.4.6-trinitrophenol. The direction in which the mean value has been shifted on transition from the lower to the higher level of pH can be found in Table 5 or more quickly in Fig. 4 (p. 492). Here the mean values of the thresholds of the group have been plotted on a logarithmic scale for every actual substance and for the two levels of pH studied. As will be seen, a remarkable drop in the threshold was obtained only for *p*-nitrophenol at the change from pH 5.1 to 7.8. These results will be considered in connection with the discussion regarding the results obtained in the statistical treatment of the threshold values of the observers in group I on p. 501.

B. *Observers in group I*

The standardized method of testing has been elaborated, with the aim of keeping the chance variations of the thresholds of taste of the two observers upon the same level. Only the factors that could be imagined to affect the level of the thresholds (quality of water and temperature) were varied.

The data collected under such conditions, when observer, quality of water, and temperature were considered at two levels, have been statistically treated as a

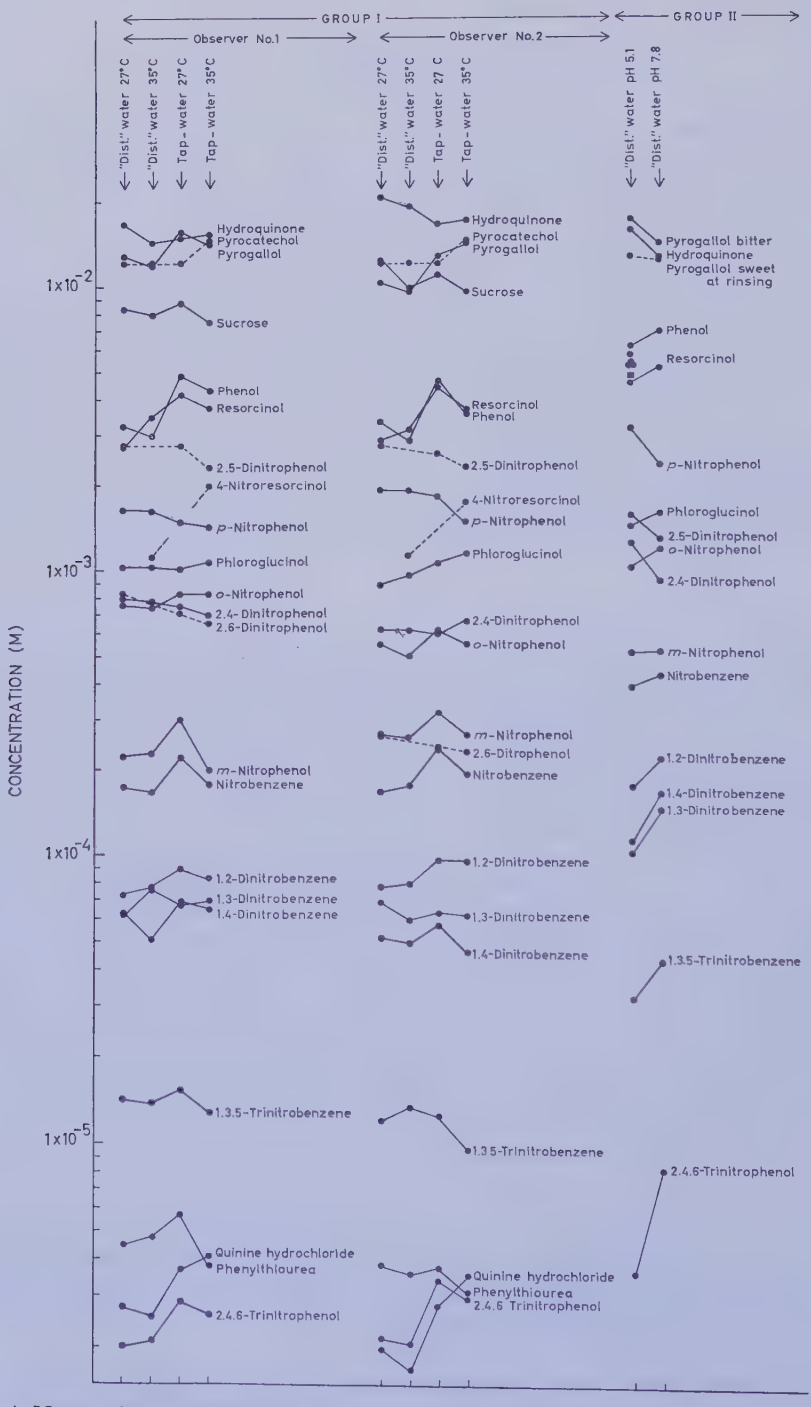

Fig. 4. Mean values of threshold of observers in group I and of group II as a whole obtained for the compounds under the actual experimental conditions. It should be noted that adjacent points representing mean threshold values for the same compound under the different experimental conditions in question are connected by lines *only* in order to make the diagram more easy to survey.

Table 15. Results of the analysis of variance of the threshold values of group I obtained for *m*-nitrophenol.

The P-values in cols. 6, 11 and 16 are the probabilities of obtaining such large F-quotients as those found on the corresponding lines in cols. 5, 10 and 15, respectively. The symbols < and > =smaller than and greater than. Superscripts ***, ** and * at a P-value for an actual factor mean that the hypothesis ("this factor does not affect the magnitude of the absolute thresholds") has been rejected at the levels of 0.001, 0.01 and 0.05 resepectivoly. When there is no superscript, the hypothesis is not rejected.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Treatment:														
	2 × 2 Factorial, Observer No. 1					2 × 2 Factorial, Observer No. 2					2 × 2 × 2 Factorial, Observers Nos. 1 and 2				
Source of variation	Sum of squares	Degrees of freedom	Mean squares	F	P	Sum of squares	Degrees of freedom	Mean squares	F	P	Sum of squares	Degrees of freedom	Mean squares	F	P
Between observers, O											1.5445	1	1.5445	11.4	< 0.01*
Between kinds of water, W	.2928	1	.2928	1.91	> 0.05	.4263	1	.4263	3.64	> 0.05	.7129	1	.7129	5.28	< 0.05*
Interaction O × W											.0063	1	.0063	.046	> 0.05
Between temperatures, T	1.1234	1	1.1234	7.34	< 0.05*	.4743	1	.4743	4.05	> 0.05	1.5288	1	1.5288	11.3	< 0.01*
Interaction O × T											.0689	1	.0689	.510	> 0.05
Interaction W × T	1.4258	1	1.4258	9.31	< 0.01**	.2333	1	.2333	1.99	> 0.05	1.4063	1	1.4063	10.4	< 0.01**
Interaction O × W × T											.2528	1	.2528	1.87	> 0.05
Experimental error	2.4504	16	.1532			1.8738	16	.1171			4.3242	32	.1351		

2 × 2 × 2 factorial. In the same way, the data for each observer have been treated as a 2 × 2 factorial, in which the figures indicate the qualities of the water and the levels of temperature in this order.

I have no intention of entering into a detailed scrutiny of the results of the different analyses of variance. In the following pages, however, I give a detailed discussion of the analysis of variance of data obtained in experiments with *m*-nitrophenol.

Table 15 contains the figures which are of interest in the discussion. All the statistical operations were carried out on an I.B.M. data-processing computer at the Department of Physics, University of Uppsala. In accordance with the programme used, the computer gives the calculated sums of squares with six decimals. In Table 15 only the first four digits are presented.

As can be seen from cols. 5 and 6, temperature had a significant effect in the case of observer No. 1. This implies that the difference between the mean values of the thresholds obtained under the experimental conditions designated "distilled" and tap-water at 27°C and "distilled" and tap-water at 35°C (the levels of temperature are those of the epithelial surface of the tongue during the tasting, cf. p. 456), respectively,

is not of a merely accidental nature. The interaction W × T has also come into prominence. This is due to the circumstance that the effect of temperature appears with different strengths in the two qualities of water. The mean value of the thresholds is roughly the same, when "distilled" water at 27° and 35°C, respectively, are used as solvent and for rinsing. On the other hand, a pronounced fall in the thresholds results from an exchange to tap-water at these temperatures (Table 3 pp. 444–445).

In Table 15, col. 10, observer No. 2 shows the highest F-quotient for the temperature factor. The effect of this is nevertheless not sufficiently pronounced to produce significance, owing to the considerable spreading of the values for the thresholds, especially when "distilled" water was used as solvent and for rinsing.

As is obvious from cols. 15 and 16, the two observers differ from each other significantly with regard to sensitivity to *m*-nitrophenol. In Table 3 observer No. 1 exhibits under all experimental conditions much lower threshold values than observer No. 2. Otherwise the two observers react in principle in the same way to the factors studied, since evidence of interactions which refer to observers, has not appeared. This finds expression also in the circumstances (1) that the F-quotient for T is considerably greater for the group in its entirety than that for the individual observers, (2) that also the F-quotient for interaction W × T has become comparatively greater, and (3) that the tendency, observed especially in observer No. 2 but also in No. 1, towards an effect of the quality of the water has been strengthened by co-operation, with the result that significance has been reached.

With regard to the analyses of variance, it may be remarked that we have encountered *in the entire statistical material* altogether five F-quotients which are remarkably small. If in each of these cases quotients are formed by dividing the mean square of an actual factor by the mean square of the corresponding experimental error, we obtain figures of an order of magnitude which, with 16 or 32 degrees of freedom, and 1 degree of freedom, respectively, yields significance (4 cases *, 1 case ***). Against the background of the fact that altogether 234 F-quotients have been formed, I have attributed the origin of these small quotients to chance. In the same way it is probable that in particular one or more of the smaller but significant F-quotients *mean square for effect of factor/mean square for the experimental error* have attained significant magnitude due to chance.

Results and discussion of the statistical treatment

A short survey of the analyses of variance performed on the data obtained in experiments on group I is presented in Table 3 (p. 444). The kinds of factorials which have been tested for each observer are given in col. 19. For every substance the factorials containing the "observer" factor are also presented. Each of the potentially active factors and the interactions in which they can be thought to appear are shown as headings in col. 20 to 26. Three asterisks (***), two asterisks (**) and one asterisk (*) mean that the hypothesis H_0 ("this factor does not affect the magnitude of the absolute threshold") is rejected at the level of 0.001, 0.01 and 0.05, respectively. When there is no asterisk, the hypothesis H_0 is not rejected. From these symbols for the P-values we can obtain an idea of the degree of influence of the different factors, either separately or in interaction, but we get no information about the direction in which the mean value of the threshold may have been shifted on transition from one experimental condition or level to another. Information on this point is obtained

directly from cols. 4, 8, 12, and 16 or, more quickly, from Fig. 4, where the mean values of the thresholds have been plotted on a logarithmic scale for each substance. factor studied and observer.

None of the $2 \times 2 \times 2$ factorials has revealed significant evidence of interaction into which the observer enters. This implies that the two observers react essentially in the same way, when the experimental conditions in the oral cavity are varied in the way adopted in the experiments. In Fig. 4 it can also be seen that the change in the mean value of the threshold upon the change from one experimental condition to the other is very similar for the two observers. For this reason I shall not comment on the factorials for the individual observers but treat the $2 \times 2 \times 2$ factorial for the group only.

The analyses of variance have been performed for altogether 18 substances. Of these, 10 have been grouped under the denomination "sweet substances" and six under the heading "bitter substances". 2.4-Dinitrophenol and pyrogallol have been termed "remaining substances". The former proved to cause a sweet and bitter taste, while the latter was bitter. On its removal from the oral cavity, however, a sweet taste was observed (p. 478, 487).

Table 16 gives a survey of the $2 \times 2 \times 2$ factorials found in Table 3. In order to demonstrate possible similarities between the substances studied, they have been presented in the following way. The compounds making up each of the above-mentioned three groups have been arranged in four series, their order in each being determined by the tendency of the threshold mean values to be lower at the lower factor level studied, according to the size of the F-quotients found. As the lower "level" for the factors of temperature, quality of water and observer, I have chosen 27°C, "distilled" water and observer No. 1. Thus each series begins with the substance exhibiting the highest F-quotient of those having the lower threshold mean value at the lower level of the factor studied, and ends with a substance which shows the highest F-quotient at the higher level. The other substances in the group in question are thus arranged so as to represent the gradual transition between these extremes.

1 *Effect of the "observer" factor*

The observed circumstance, that the thresholds of the observers in group I differ significantly from each other as regards certain of the sweet substances tested (observer No. 1 being more sensitive than No. 2 to the first four substances, but observer No. 2 being more sensitive to the last compound mentioned in Table 16. col. 2*c*), while, as regards others, the observers show about the same acuity, may be interpreted as indicating that in the cases in question different populations of receptors and/or different types of receptor sites have been stimulated. These could for genetic or other reasons be found in the different observers in different proportions. The observation of the circumstance that the absolute thresholds of one observer are lower than or the same as those of the other observer for all substances does not, of course, entitle us to assume that the substances affect the same type of receptors and/or receptor sites. It is conceivable that these receptors are of different kinds, but occur in the same proportions in both of the observers.

2 *Effect of the factors of temperature and water quality*

Sweet substances. Table 17 (p. 498) shows again the results of the actual analyses of variance. In principle the substances have been arranged in the descending order of active factors.

Table 16. Effect of the factors studied on the size of the mean threshold

The compounds are arranged in order in the way described on p. 495. The meaning of the superscripts W × T are explained

1	2			3	
	a	b	c	a	b
	Factor involved:				
	O			W × T	
	F-quotient on occasion when the mean threshold value was lower at:				
Group	Lower level tested (O. No. 1)	Higher level tested (O. No. 2)	Compound	F-quotient	Compound
Sweet compounds	42.6***		Sucrose	12.3**	Resorcinol
	21.4***		Hydroquinone	10.4**	*m*-Nitrophenol
	11.4 **		*m*-Nitrophenol	4.40*	Nitrobenzene
	9.92**		*p*-Nitrophenol	3.23	Hydroquinone
	3.04		1.2-Dinitrobenzene	1.46	*p*-Nitrophenol
	.633		Nitrobenzene	1.35	Phenol
	.005		Resorcinol	.772	1.2-Dinitrobenzene
		.160	Phloroglucinol	.173	Phloroglucinol
		1.25	Phenol	.002	Sucrose
		50.6***	*o*-Nitrophenol	.001	*o*-Nitrophenol
Bitter compounds	2.55		2.4.6-Trinitrophenol	13.1**	Phenylthiourea
		1.75	1.3-Dinitrobenzene	7.64**	Quinine hydrochloride
		12.8**	1.3.5-Trinitrobenzene	6.79*	1.3.5-Trinitrobenzene
		21.8***	1.4-Dinitrobenzene	1.11	2.4.6-Trinitrophenol
		26.7***	Quinine hydrochloride	.089	1.4-Dinitrobenzene
		40.9***	Phenylthiourea	.055	1.3-Dinitrobenzene
Remaining compounds		9.15**	Pyrogallol	.594	Pyrogallol
		12.0 **	2.4-Dinitrophenol	.083	2.4-Dinitrophenol

With regard to the first three substances, it appears that the effect of temperature makes itself felt with different intensities in waters of different qualities and vice versa, i.e. the effect of the quality of the water is different at different levels of temperature. In this respect these three substances form a group by themselves. The next two substances, viz. phenol and sucrose, act similarly in so far as in both the effect of temperature makes itself felt, while in the testing of sucrose the factor embodied in the quality of the water does not seem to have any effect.

In the last five substances, only the effect of the quality of the water is apparent. Otherwise the group is heterogeneous, since the thresholds obtained for 1.2-dinitrobenzene, phloroglucinol, and *o*-nitrophenol rise at the transition from "distilled" water to tap-water, while those for hydroquinone and *p*-nitrophenol decrease under corresponding conditions.

Bitter substances. The bitter substances are shown in the same way in Table 17.

values for the compounds tested according to the $2 \times 2 \times 2$ factorials.

***, **, * and no asterisk adjoining the F-quotients is explained on p. 494. The symbols O, W, T and in Table 15.

4			5		
a	b	c	a	b	c

W			T		
F-quotient on occasion when the mean threshold value was lower at:			F-quotient on occasion when the mean threshold value was lower at:		
Lower level tested ("dist." w.)	Higher level tested (tap-w.)	Compound	Lower level tested (27°C.)	Higher level tested (35°C.)	Compound
38.6***		Phenol	1.48		Phloroglucinol
37.5***		Resorcinol	.000		1.2-Dinitrobenzene
14.0***		1.2-Dinitrobenzene		.097	Resorcinol
11.4**		Nitrobenzene		1.06	Hydroquinone
5.28*		*m*-Nitrophenol		1.07	*o*-Nitrophenol
4.75*		*o*-Nitrophenol		2.14	*p*-Nitrophenol
4.68*		Phloroglucinol		4.51*	Nitrobenzene
	1.10	Sucrose		8.62**	Phenol
	6.43*	Hydroquinone		11.3**	*m*-Nitrophenol
	7.54**	*p*-Nitrophenol		11.6**	Sucrose
71.7***		Quinine hydrochloride	1.31		Quinine hydrochloride
29.8***		2.4.6-Trinitrophenol	.205		1.3-Dinitrobenzene
5.84*		1.4-Dinitrobenzene		1.11	2.4.6-Trinitrophenol
	.124	1.3-Dinitrobenzene		3.50	1.3.5-Trinitrobenzene
	.135	Phenylthiourea		9.58**	1.4-Dinitrobenzene
	1.90	1.3.5-Trinitrobenzene		12.0**	Phenylthiourea
24.5***		Pyrogallol		.003	2.4-Dinitrophenol
	.489	2.4-Dinitrophenol		.452	Pyrogallol

Phenylthiourea, quinine hydrochloride, and 1.3.5-trinitrobenzene exhibit the effect of the W × T interaction. The group is, however, heterogeneous with regard to the effect of temperature and the quality of the water. The similarity between the other bitter substances is not particularly great either. The effect of the quality of the water is very pronounced as regards 2.4.6-trinitrophenol, but less pronounced as regards 1.4-dinitrobenzene. The latter exhibits in addition an effect of temperature. In 1.3-dinitrobenzene, lastly, no significance is obtained for any of the factors studied. From Fig. 4 one may have expected to find evidence of an interaction into which the observers entered, since they seemed as a rule to react in opposite ways to changes of the quality of the water and temperature. The difference was, however, small, while at the same time the spreading of the thresholds in question was considerable. For this reason the F-quotient falls very short of a magnitude that would suffice for significance.

Table 17. Effect of the factors W and T on the size of the mean threshold value for the compounds tested according to the $2 \times 2 \times 2$ factorials.

The compounds are arranged in descending order of the number of active factors. The meaning of the symbols ***, **, *, and no asterisk in cols. 2–4 is explained on p. 494. The signs + and − next to the symbol for the value of P in cols. 3 and 4 indicate that at the transition from 27° to 35°C (col. 3) or from "distilled" water to tap-water (col. 4) a rise or a fall respectively in the mean value of the thresholds occurred.

1	2	3	4
Compound	Interaction W × T	Between T	Between W
Nitrobenzene	*	*−	**+
m-Nitrophenol	**	**−	*+
Resorcinol	**		***+
Phenol		**−	***+
Sucrose		**−	
1.2-Dinitrobenzene			***+
Phloroglucinol			*+
o-Nitrophenol			*+
Hydroquinone			*−
p-Nitrophenol			**−
Phenylthiourea	**	**−	
Quinine hydrochloride	**		***+
1.3.5-Trinitrobenzene	*		
1.4-Dinitrobenzene		**−	*+
2.4.6-Trinitrophenol			***+
1.3-Dinitrobenzene			
Pyrogallol			***+
2.4-Dinitrophenol			

Remaining substances. Only the change of the quality of the water seems to have affected the value of the thresholds for pyrogallol. For 2.4-dinitrophenol, lastly, no factor apart from "the observer" reached significance.

Hahn, Kuckulies & Taeger (1938) determined the magnitude of the absolute threshold at different temperatures, for example, 19° and 32°C, for a number of sweet and bitter substances, including nitrobenzene, phloroglucinol, resorcinol, quinine hydrochloride, and 2.4.6-trinitrophenol. In determining the thresholds, the solutions were passed over a limited part of the tongue. Distilled water was used as solvent and for rinsing and the application of the stimuli lasted 6–7 seconds. The temperature of the epithelium can be considered to have changed very little in the course of an experiment. For most of the *sweet* substances the authors noted falls of varying extent in the threshold in question, when the temperature at the epithelial surface changed from 19° to 32°C. With corresponding changes of the experimental conditions, on the other hand, they generally found for the majority of the *bitter* substances, a decided, although slight rise of the threshold.

The present results differ in several respects from those achieved by Hahn *et al.* Thus, for resorcinol and phloroglucinol ("distilled" water) I found no fall in the absolute thresholds upon the change from the lower to the higher level of temperature, while the above-mentioned authors found for resorcinol a particularly strong effect

of the temperature upon the threshold. With regard to the bitter substances, the changes of the thresholds which I observed may be designated as falls. The above-mentioned divergences may be due to (1) differences between observers as to the way in which the sense of taste functions, (2) the methods used, and/or (3) the quality of the water used as solvent and for rinsing. It is impossible to decide which of these possible explanations is the most probable, amongst other things, on account of the fact that Hahn *et al.* supplied no information about the pHs of the tested solutions nor about the quality of the distilled water used as solvent and for rinsing. Judging from the significant $W \times T$ interaction obtained in the present investigation, the effect of the temperature is in certain cases of different intensity in different qualities of water. This has already been pointed out above.

The temperature of the epithelial surface of the tongue in the experiments of the present investigation is 8–10°C higher, when the temperature of the introduced liquids is *c.* 36.9°C than when it is *c.* 20.4°C. According to the Q_{10} rule, a doubling of the speed of reaction can be expected in enzymatically determined processes, if the temperature is raised by 10° within the range 20–40°C. In 1950 Kistiakowsky propounded the hypothesis that the sense of smell is dependent upon enzymatically conditioned reactions. In the papillae foliatae of the rabbit Baradi & Bourne (1951) found two enzymes which were inhibited by certain taste substances but not by others, and which for this reason could be imagined to play a decisive role in the process of stimulation. It is possible that at least some of the processes involved in the transformation of the depolarization of a receptor cell into a nerve impulse may be enzymatically conditioned. It is hardly necessary to point out that they must also be more or less intimately linked up with the turnover of energy in the cell. Thus, provided also that the hypothesis propounded by Baradi and Bourne is not valid, an effect of the temperature may be expected.

Judging by the experiments discussed in the present work, the dependence of the thresholds upon the temperature is on the whole not very pronounced. The greatest displacement of the mean value of the threshold was observed in observer No. 1 for the substances *m*-nitrophenol and phenylthiourea, when tap-water was used both as solvent and for rinsing. The coefficient of temperature (Q_{10}) was in either case about 1.88, calculated on a temperature interval of 8°C and treating the threshold value as a coefficient for a rate of reaction—possibly a reaction between the stimulus and the excitable system.

Yamashita *et al.* (1964) found in the course of electrophysiological experiments on cats that the response in the chorda tympani was greatest when the tongue was stimulated, within the interval 30–35°C, by a 0.5 M solution of NaCl or KCl and by a 5×10^{-3} M solution of quinine or HCl, respectively. For the responses "immediately after the stimulation" with NaCl, KCl, HCl and quinine, they found, within the temperature interval 10–20°C, temperature coefficients of 1.54, 1.92, 3.46 and 2.34 respectively. Within the interval 15–25°C the corresponding figures were 1.38, 1.43, 1.76, and 1.93 and within the interval 35–45°C −1.70, −1.31, −2.38 and −3.60. Thus, an effect of a change of temperature does not appear in the middle of the temperature interval examined. This can also be seen, for example, in Fig. 4 of the report presented by Yamashita *et al.* According to Sato (1963), most of the individual taste units in the cat are also able to respond to temperature changes. Thus, Ringer solutions at temperatures higher or lower than 30°C initiate responses in single-fibre preparations sensitive to quinine, HCl, NaCl or water. In the experiments described above, Yamashita *et al.* adapted the temperature-sensitive elements, including the

taste units, to a certain temperature before the taste stimulations were performed, in order to eliminate the temperature responses. In this connection it would be of interest to know whether or not such an adaption of the taste units is of importance, as regards their responsiveness to other kinds of stimuli applied meanwhile. If the units have a lower responsiveness to one kind of stimulus when adapting or adapted to another, results such as those obtained by Yamashita *et al.* may be expected. We must take into account the possibility that human taste receptors function in a similar way to those of the cat, according to Yamashita *et al.* Thus, if the response of the actual receptors of the observers in group I was greatest in the region between the two levels of temperature studied, this would explain the comparatively low effect of temperature obtained in the experiments.

It must also be stressed that the receptors for cold are stimulated at the lower level of temperature studied. The presence of a simultaneous sensation of cold on tasting the liquids may result in the observers finding it increasingly difficult to perceive the vague sensations caused by the substances in the neighbourhood of the absolute threshold. Other factors may also be though of as furthering an effect of temperature. A change of temperature, for example, may alter the ease with which hydrophobic bonds are formed in different parts of the excitable system, and at the same time also its hydration, structural state and readiness to react when stimulating molecules are applied (compare the effect of temperature on the equilibrium between the sol and gel states of gelatin for instance).

As can be seen from Table 16 and the synoptic Table 17, the effect of the quality of the water made its appearance in the majority of the factorials, either directly or hidden in the $W \times T$ interaction. Exceptions from this are the factorials for the substances sucrose, 1.3-dinitrobenzene, and 2.4-dinitrophenol. Here I wish to call the reader's attention to the fact that a significant *fall* in the threshold at the change from the "distilled water" level to the "tap-water" level was obtained only in the cases of hydroquinone and *p*-nitrophenol.

According to p. 491 and Table 5 (pp. 450–451), col. 12, a significant difference in magnitude between the thresholds is apparent for only five of the 16 substances tested by group II (1.2-, 1.3-, and 1.4-dinitrobenzene, *p*-nitrophenol, and 2.4.6-trinitrophenol). A fall in the threshold was obtained only for *p*-nitrophenol at the transition from the lower to the higher level of pH (cf. the results of the analyses of variance performed upon the data obtained in the experiments with group I).

The question arises, whether the difference between the content of ions, the concentration of unprotolyzed or protolyzed forms of the substance, and/or the concentration of hydrogen ions in the solutions prepared with "distilled" water and tap-water, respectively (tasted by group I) or "distilled" water of pH 5.1 and pH 7.8 (tasted by group II) may be thought to have contributed to the effect of the unspecified factor which has been designated as "quality of the water" in the statistical analyses.

I have previously pointed out (p. 456) that all the liquids tasted may be considered as unphysiological solutions, in the sense that they differ markedly from the saliva with regard to the type and content of ions occurring in them. While, according to Table 50 in Spector's *Handbook of Biological Data*, the concentrations in the saliva of the ions of sodium, potassium, calcium and chlorine, to which great importance is attributed for the maintenance of the potential of the cell membrane, are about 24000, 18000, 1400, and 15500×10^{-6} M respectively, the corresponding quantities in tap-water according to the information given in Table 7 (p. 457) and on p. 458,

are 1000, 200, 3000 and 700–900 $\times 10^{-6}$ M. Thus, only the concentration of calcium is about the same in both cases. In the majority of the liquids containing threshold concentrations of the different substances dissolved in distilled water, the concentrations of the above-mentioned ions are still lower, even after mixing with the saliva accumulated in the oral cavity (Table 9 (pp. 461, 463), col. 24; see also p. 464).

Harris & Kalmus (1949) and Bourlière *et al.* (1958) are of the opinion that distilled water has a taste which they call "unaccustomed" and "particulière", respectively. This should not be assumed to imply that the sensitivity of certain receptors is augmented for some reason in a practically ion-less environment, to such a degree that a spontaneous activity is initiated, since Zotterman (1959) has been able to show that to man water is tasteless. However, the fact that with the observers in group I the absolute thresholds obtained were as a rule lower, when the substances were dissolved in distilled water than when tap-water was used as solvent, may then be explained by the assumption that the sensitivity of the taste receptors was greater in media with very low contents of ions. Admittedly, as has already been mentioned, no effect of the quality of the water could be observed in the single case studied (2.4-dinitrophenol), in which the concentration of sodium-ions was about the same in samples of liquids made up with distilled water and tap-water (see Table 9, col. 24). Yet the circumstance that the thresholds for hydroquinone and *p*-nitrophenol were lower, when tap-water was used as solvent than when the substances were dissolved in distilled water cannot be explained in the above-mentioned way. Furthermore the actual thresholds of the observers in group II proved in a number of cases to be *significantly lower* at the lower pH level (*p*-nitrophenol forming an exception), in spite of the fact that the content of ions was quite inconsiderable in both types of preparations (Table 9, col. 25). On the other hand, in cases in which the sodium-ion concentration was markedly greater at pH 7.8 (for example, in the cases of pyrogallol, phloroglucinol and *o*-nitrophenol) no effect of pH was observed.

The majority of the protolyzing sweet substances appear at both pH levels mainly in unprotolyzed form (cf. Table 9, cols. 7, 13, 19 and 23 and p. 468). Yet in tap-water the number of ions of *o*- and *p*- nitrophenol predominates over that of the corresponding unprotolyzed molecules (cf. Table 9, col. 13). While, however, the threshold for *p*-nitrophenol and hydroquinone of the observers in group I falls at the change from the lower to the higher pH level, it rises significantly for the other sweet substances, with the exception of sucrose. In the cases (1.2-dinitrobenzene, *p*-nitrophenol), in which the pH can be shown to have had an effect upon the magnitude of the thresholds of the observers in group II, the tendency was the same as in group I (cf. Fig. 4).

The effect of the quality of the water is evident for the bitter substances quinine hydrochloride, 1.4-dinitrobenzene, and 2.4.6-trinitrophenol in group I and for 1.3- and 1.4-dinitrobenzene and 2.4.6-trinitrophenol in group II. Only the last-mentioned substance occurred almost entirely protolyzed in the solutions at the tasting.

Finally, a fall in the absolute threshold was obtained for the "remaining" substances (group I) at the transition from the lower to the higher level only in the case of the slightly protolyzing substance pyrogallol (cf. Fig. 4).

The effect of the quality of the water may appear both with unprotolyzed and protolyzed substances. This effect is not particularly great in the cases in which a considerable change from the uncharged to the charged form of the stimulating molecules took place at the change from the lower to the higher level, as was the case

with the mononitrophenols. The charge seems to be of small importance for the effect of the quality of the water. The cause has to be looked for elsewhere. It is evidently conceivable, that the change in pH at the transition from "distilled" to tap-water brought about changes in some physicochemical property of the stimulating substances, a property not taken into account here but of importance in the process of stimulation. The change in pH must, however, also have affected the properties of the receptor membrane which are probably important for this process. According to modern views, a cell membrane is formed by a mosaic of proteins and lipids, in which both proteins and lipids possess acid-base properties. The number and kind of polar groups present, as well as the composition, properties and structural arrangement in the biological surface, are determined by genetic inheritance and perhaps also to a certain extent by the age of the receptor cells in question.

The polar groups of the biological surface, which are supposed to be directed towards the water phase, may constitute or be a part of a receptor site or may simply be a part of the medium into which the stimulating molecules have to penetrate to reach the site of action. In such cases a shifting of the pH results in a change in the number of charged groups, changes in the stereo-structure and changes of the physicochemical properties, at least in certain parts of the biological surface. The extent of these changes depends upon the pK_a values of the protolytes in question. The circumstance that a rise of pH in the oral cavity results in a fall of the threshold for hydroquinone and *p*-nitrophenol but in a rise of the threshold for the other sweet-tasting substances, may indicate the occurrence of different kinds of receptor sites in populations of receptors which are connected with centra for sweet taste. Furthermore the fact that certain observers exhibited a low sensitivity to phenylthiourea but normal sensitivity to other bitter compounds and the observation that the acuity of observer No. 2 for *o*-nitrophenol was greater than that of observer No. 1, while the opposite was the case with regard to other sweet-tasting substances (cf. Table 16 p. 496), indicates that the importance of the different kinds of receptor sites varies, probably on account of their number, from individual to individual.

Finally, the fact that the W × T interaction is obtained for the compounds resorcinol, nitrobenzene, and *m*-nitrophenol may indicate that the component or components in the receptor cells which takes part in the initial reaction with these substances has a pK_a value in the neighbourhood of one of the pH regions examined and thus in that condition is considerably less polar than in the other. If the stimulating, polar molecule has to enter into a low polar medium before reaching the site of action, an increase in kinetic energy would be favourable for the rate of penetration (cf. p. 428). At the other pH level in question the effect of temperature should be less pronounced, as the medium is polar in nature.

The relatively small magnitude of the effect of temperature argues against the assumption that the stimulation of a receptor is the direct result of enzymatic processes. For the reasons given above (pp. 499–500), the dependence of the thresholds upon temperature should be studied within a wider range of temperature before a final judgement is passed.

VIII. The connection between the intensity of the sensation and the strength of the stimulus for some sweet and bitter substances

I pointed out by way of introduction (p. 426) that the absolute threshold for a compound refers to the lowest concentration necessary to produce a sensation. It is not out of the question that substances which cause identical taste sensations influence the excitable system in the receptors in different ways, for example, by influencing to a greater or lesser extent the speed of the chemical reactions which are of primary or secondary importance in the creation of the nervous impulses. A certain relative increase from the threshold level of the strength of stimuli which differ considerably in the above-mentioned way will probably not always result in an equal increase of the intensity of sensation. The latter may be expected to be a function of the total frequency of discharge in the taste fibres. If it can be shown that the relative stimulating abilities of the substances at the threshold levels differ from that related to a definite and higher level of the intensity of sensation, this fact will definitely indicate differences in the modes of action of such compounds. Psychophysical methods have recently been developed which permit a direct determination of the connection between the strength, S, of the stimulus and the intensity, R, of the subjective sensation for different fields of perception (cf. Guilford 1954, Björkman & Ekman 1962).

The above-mentioned type of experiment has been performed with some of the substances for which the thresholds had previously (p. 433 ff) been determined. Thus, by means of the ratio-estimation method (see p. 504), the connection between R and S has been determined for the sweet substances nitrobenzene, 1.2-dinitrobenzene, phenol, resorcinol, phloroglucinol, *o*-nitrophenol, *p*-nitrophenol, and sucrose, and for the bitter substances 1.3-dinitrobenzene, 1.3.5-trinitrobenzene, 2.4.6-trinitrophenol, quinine hydrochloride, and phenylthiourea. For the substances 1.3.5-trinitrobenzene, 2.4.6-trinitrophenol, sucrose, quinine hydrochloride and phenylthiourea, the connection between R and S has been determined also by a procedure called the ratio-calculation method, which with respect to the technique of scaling agrees with that used in the application of the ratio-production method (see p. 507).

In each of the functions obtained, R is expressed in arbitrary units. In order to permit approximate comparisons between the stimulating abilities of the sweet substances and between the stimulating abilities of the bitter substances at different levels of the intensity of sensation, the R values of the individual scales should be standardized. This has been accomplished by searching, with the aid of the magnitude-estimation method (see p. 510) within series of concentrations of sucrose and quinine hydrochloride, for *a point of subjective equality* (P.S.E.) representing a concentration of each of the sweet and bitter substances, respectively. Each of these concentrations can thus be referred to a certain point upon the curves for the R-S functions obtained for sucrose or quinine hydrochloride by the above-mentioned scaling procedure (the ratio-calculation method). The R values have been standardized by graphically drawing each of the R-S functions of the substances through the P.S.E. in question. In this way the values for R in the scales for sweet and bitter substances have been expressed automatically in units representing the R values found for sugar and quinine hydrochloride, respectively.

The advantages, disadvantages, and risks in the practical and theoretical applications of a number of different psychophysical methods have been discussed by Björkman & Ekman (1962). The ratio-production method has been used by Lewis

(1948) in the construction of psychological scales of taste. This method, and the ratio-estimation and magnitude-estimation methods, have been tested by Engen (1961) and Engen & Lindström (1962) for the direct scaling of the intensity of smell.

Experimental conditions

Distilled water (electric conductivity $2.5–3.3 \times 10^{-6}$ ohm^{-1} cm^{-1}) was used as solvent and for rinsing in all the experiments described below. In all cases the preparation of stock solutions and the checking of the concentrations was performed as described above (p. 440). Solutions and rinsing water were adjusted before the tasting to a pH of 5.1 by the addition of suitable amounts of HCl or NaOH. Apart from those of 2.4.6-trinitrophenol and possibly also those of phenylthiourea and quinine hydrochloride (pK_a values not found in literature, cf. p. 463), the molecules of the compounds appeared mainly in undissociated form in the solutions. After being tasted, these further showed about the same concentrations of Na^+, Cl^-, K^+, and Ca^{++} respectively (figures not given). The temperature of the room was in all cases between 22° and 23°C.

Methods

In the course of the experiments described below, only one observer, viz. the author, was tested. For three weeks preceding the start of the experiments the observer trained himself to carry out assessments according to the principles laid down for the different methods applied. At the same time different ways of presenting the combinations of stimuli in the tasting were tested. The mode of presentation may have been of importance for the results of the assessments.

In order to smooth out any progressive effects which may occur in cases in which the two stimuli under comparison have to be presented in succession, it is possible to present the standard first and the comparison solution last in one half of the cases and vice versa in the other half. However, this procedure entails rinsings between the tasting of the first and second liquid in each pair. This is necessitated by the circumstance that an effect of addition (for the definition of this term, see p. 449) may be expected to appear, if the stronger concentration is tasted first. The more thoroughly this effect of addition is eliminated, i.e. the greater the number of the rinsings carried out by the observer before the tasting of solution No. 2, the more diffuse will be the memory of the intensity of the sensation experienced at the tasting of the first sample. This leads to an impairment of the reproducibility and thus to increased variation of the estimations obtained. On the other hand, the weaker solution in each pair may be tasted first without any attempt to smooth out possible progressive effects. In this case sample No. 2 can be taken immediately after the discharge of the first solution tasted. This procedure has, amongst other things, the advantage that the observer is able to compare almost simultaneously the intensities of sensation of the two stimuli, and that the influence upon the assessment of the centrally localized processes which are associated with memory are reduced to a minimum. Whenever possible, the author applied this procedure in this investigation.

1. *Ratio-estimation method*

The analysis of three preliminary experiments carried out under identical conditions but on different days and at the beginning of this part of the investigation, i.e.

while the observer could still be regarded as untrained, indicated that the parameters entering into the *R-S* function were not quite stable. In order to eliminate as much as possible such variations, which might become of importance if the experiments were to extend over a longish time, it was decided that all substances with similar tastes should be tested at the same tasting and within the shortest possible space of time. For this purpose *three* solutions of different molarities were prepared for each substance. These concentrations formed a geometrical series, in which the lowest concentration was about 2.5 times stronger than that representing the absolute threshold of the observer for the substance, according to the previously performed determinations (cf. p. 444). The steps of the concentrations in the series for nitrobenzene and 1.2-dinitrobenzene were only 0.25 log units because of the small interval between the absolute threshold and the concentration of saturation. For the other substances the steps were 0.5 log units (cf. Table 18 p. 506, cols. 7 and 8).

The solutions included in the geometrical series of an actual compound were poured into brown test-tubes and then combined in pairs, so that four pairs of each possible combination were obtained. As no combination was allowed to contain two identical stimuli, altogether twelve pairs of each compound were obtained. Pairs belonging to substances with the same taste quality were then combined to form a collection of pairs and were presented to the observer in random order. The strongest solution in each pair, (which was taken immediately after the discharge of the first one), was regarded as the standard stimulus and was given the arbitrary value 100 by the observer. The task then consisted in expressing the strength of the weaker solution as a percentage of the stronger. Before the tasting of the solutions in each pair, the observer performed four rinsings. Four rinsings followed upon the assessment of the last pair. The experiments just described were repeated once more, the second start being made about 1 hour after the termination of the first experiment. Now the random arrangement was the same as in the first experiment, but the series of pairs obtained was presented in reverse sequence. In this way altogether eight estimations were obtained for each combination. The results of the estimations were entered by the observer in a journal prepared for the purpose. The procedure applied here was otherwise just the same as that used for the determination of the thresholds, the only exception being that no rinsings were intercalated between the samples belonging to a pair.

Solutions and rinsing water had temperatures of 21.3° and 21.7°C, respectively, when the sweet and the bitter substances were tasted.

One day each was devoted to the sweet and the bitter substances.

Results. Since no combination was allowed to contain two identical stimuli (cf. Björkman and Ekman 1962), these experiments resulted in altogether 24 ($=3\times8$) estimations for most of the substances. In three cases (phenol, resorcinol and phloroglucinol), only 23 estimations were obtained, as one combination was not judged by the observer during the tasting. The arithmetical means and the standard deviations of the logarithmic values for the ratio estimations are shown in Table 18, cols. 4–5, for each substance and each combination of stimuli (col. 2). As can be seen, the standard deviations oscillate around the value 0.1. The antilogarithms of the three above-mentioned mean values (col. 6) for each substance were then entered in a ratio matrix (not shown here), and the scale values for the series of stimuli given in cols. 7 and 8 for each compound were calculated (cols. 9–11) according to the method developed by Ekman (1958).

Eisler (1960) has proposed a method of studying the internal consistancy of a

Table 18. Ratio-estimation data obtained from observer No. 1.

Abbreviations: S_1, S_2 and S_3 – the weakest, the medium and the strongest stimulus presented, n = number of ratio estimations obtained for the actual pair of stimuli, $\bar{X}_{\log \text{r.e.}}$ and $s_{\log \text{r.e.}}$ = the arithmetical mean and standard deviation respectively of the ratio estimates (r.e.) in question.

1	2	3	4	5	6	7	8	9	10	11	12
Compound	Stimulus combination	n	$\bar{X}_{\log \text{r.e.}}$	$s_{\log \text{r.e.}}$	Antilog $\bar{X}_{\log \text{r.e.}}$	Stimulus S_x	Conc. S_x $\times 10^6$ M	Scale value, R	Scale value expressed as RS_x/RS_1	log RS_x/RS_1	Standardized log scale values
Nitrobenzene	S_1–S_2	8	−.3014	.1058	.499	S_1	435	.472	1.000	.0000	.32
	S_1–S_3	8	−.6699	.1216	.214	S_2	774	1.005	2.128	.3280	.65
	S_2–S_3	8	−.2957	.1041	.506	S_3	1,380	2.110	4.466	.6499	.97
1.2-Dinitrobenzene	S_1–S_2	8	−.2938	.0956	.508	S_1	181	.423	1.000	.0000	.22
	S_1–S_3	8	−.8011	.1941	.158	S_2	322	.938	2.217	.3460	.57
	S_2–S_3	8	−.3702	.1058	.426	S_3	572	2.470	5.836	.7661	.99
Phenol	S_1–S_2	8	−.4321	.0783	.370	S_1	8,030	.384	1.000	.0000	.45
	S_1–S_3	7	−.8220	.1439	.151	S_2	25,400	1.086	2.830	.4518	.90
	S_2–S_3	8	−.3365	.0860	.461	S_3	80,300	2.464	6.419	.8075	1.25
Resorcinol	S_1–S_2	8	−.6897	.1641	.204	S_1	6,780	.259	1.000	.0000	.20
	S_1–S_3	8	−1.1197	.1396	.076	S_2	21,400	1.166	4.502	.6534	.85
	S_2–S_3	7	−.5241	.1100	.299	S_3	67,800	3.568	13.774	1.1391	1.34
Phloroglucinol	S_1–S_2	8	−.3459	.1056	.451	S_1	2,580	.483	1.000	.0000	.44
	S_1–S_3	8	−.6076	.1124	.247	S_2	8,160	1.057	2.188	.3301	.77
	S_2–S_3	7	−.2785	.1362	.527	S_3	25,800	1.977	4.091	.6118	1.05
o-Nitrophenol	S_1–S_2	8	−.3433	.0704	.454	S_1	1,890	.411	1.000	.0000	.53
	S_1–S_3	8	−.8005	.1204	.158	S_2	5,980	.920	2.236	.3495	.88
	S_2–S_3	8	−.4409	.1532	.362	S_3	18,900	2.575	6.262	.7967	1.33
p-Nitrophenol	S_1–S_2	8	−.4161	.0855	.384	S_1	4,100	.431	1.000	.0000	.37
	S_1–S_3	8	−.6829	.1228	.203	S_2	13,000	1.028	2.385	.3775	.75
	S_2–S_3	8	−.3832	.1065	.414	S_3	41,000	2.272	5.267	.7216	1.09
Sucrose	S_1–S_2	8	−.4702	.1167	.339	S_1	20,800	.357	1.000	.0000	.38
	S_1–S_3	8	−.8807	.1360	.132	S_2	65,800	1.122	3.141	.4971	.88
	S_2–S_3	8	−.3354	.0785	.462	S_3	208,000	2.596	7.266	.8613	1.24
1.3-Dinitrobenzene	S_1–S_2	8	−.3937	.0966	.404	S_1	155	.469	1.000	.0000	.66
	S_1–S_3	8	−.6052	.1145	.248	S_2	490	1.159	2.471	.3929	1.05
	S_2–S_3	8	−.2139	.0727	.611	S_3	1,550	1.894	4.038	.6062	1.27
1.3.5-Trinitrobenzene	S_1–S_2	8	−.2877	.1190	.515	S_1	35.5	.485	1.000	.0000	.76
	S_1–S_3	8	−.6479	.1082	.225	S_2	112	1.016	2.094	.3210	1.08
	S_2–S_3	8	−.2681	.0942	.539	S_3	355	2.034	4.191	.6223	1.58
2.4.6-Trinitrobenzene	S_1–S_2	8	−.3347	.0756	.463	S_1	5.03	.470	1.000	.0000	.75
	S_1–S_3	8	−.5609	.1015	.223	S_2	15.9	1.082	2.304	.3625	1.11
	S_2–S_3	8	−.2380	.0849	.578	S_3	50.3	2.001	4.267	.6201	1.37
Quinine hydrochloride	S_1–S_2	8	−.3310	.0908	.467	S_1	6.88	.493	1.000	.0000	.78
	S_1–S_3	8	−.5944	.0554	.254	S_2	21.8	1.084	2.198	.3420	1.13
	S_2–S_3	8	−.2320	.0739	.586	S_3	68.8	1.900	3.854	.5859	1.37
Phenylthiourea	S_1–S_2	8	−.3352	.1118	.462	S_1	11.2	.456	1.000	.0000	1.05
	S_1–S_3	8	−.6862	.0901	.206	S_2	35.4	1.055	2.312	.3640	1.42

ratio matrix. According to him, an estimated mean value of the ratios can be reproduced $n-2$ times (where n is the number of stimuli) by pairs of other estimated mean values. As three mean values of the ratios have been obtained for each substance tested, each estimate can be reproduced only once. This, of course, weakens the reliability of the test of consistency. When, however, the estimate for the stimulus combination $S_1 - S_2$ is reproduced, the value obtained for each of the tested substances falls well within the limits of the standard deviation around the mean of the estimated ratio in question. This test thus supplies no argument for inconsistency. There is nevertheless a trend in this direction although it is a weak one. This is obvious from the fact that the reproduced value is in most cases lower than the experimentally obtained value, when all sweet and/or bitter substances are considered together. The observer seems to have overrated the ratios between S_1 and S_3, and/or underestimated the ratios between S_1 and S_2 and between S_2 and S_3. This is in fact the conclusion arrived at by Eisler from his experiments with weights. The data in Table 18 are, however, presented without the corrections used by Eisler.

In preliminary experiments, using the ratio-estimation method, sodium nitrite was also tasted. By mistake the concentrations $3.00\times$, $9.49\times$, and 30.0×10^{-2} M were used, which were stronger than intended. When the pairs into which the last-mentioned concentration entered were tasted, the estimations obtained were "infinity", indicating that only the stronger solution had no sweet taste. Thus the substance causes a sweet taste only within a relatively restricted range of concentration above the threshold of recognition (*c.* 2.25×10^{-3} M) and agrees in this respect with several of the salts studied by Renqvist (1919). The above conclusion is also supported by the circumstance that the sweet taste was observed when the mouth was rinsed with water and the concentration of the substance during part of the period of rinsing lay within the range of concentration in question. The determination of the *R-S* function within this range is postponed to a future occasion. The observed "dependence of quality of taste on concentration" phenomenon will be discussed on p. 527.

2. *Ratio-calculation method*

Within a range of concentration above the absolute threshold, solutions were prepared of every substance tested, the concentrations, expressed in molarity, forming a geometrical series with steps of 0.1 log units. From this was selected, starting at the highest concentration, a series of standard concentrations with intervals of 0.5 log units. An exception was formed by sucrose, the difference between the standards being alternately 0.3 and 0.2 log units. In the assessment, each of the standard solutions was to be compared with the next six lower concentrations in the original series. In the course of the preliminary experiments the observer had been given the task of choosing in the assessment a stimulus which was experienced as being of half the strength of the standard (cf. Lewis (1948), Ekman (1958)). However, the observer experienced very great difficulties in indicating directly a single solution that fulfilled the requirements. These difficulties were caused by the considerable variation in the assessment.

For this reason, in the experiments reported here the observer, in assessing each pair, had to express the intensity of the sensation of the solution to be compared with the standard as a percentage of the intensity caused by the latter, according to the ratio-estimation method. The intensity of the sensation produced by the standard was given the arbitrary value 100.

All pairs containing the same standard were assembled in a collection of pairs and presented to the observer in random order. In the same way the order in which collections with different standards were to be tested was determined by randomization. The whole of this procedure was repeated three or in certain cases four times. It should be noted that the observer had a thorough knowledge of the arrangement of the experiment and of the strength and the nature of the stimuli used. Therefore a new random arrangement was made each time, in order to deprive him of the possibility of remembering the results of previously performed assessments.

Otherwise the procedure applied here was the same as that used for the determination of thresholds. The liquids presented had a temperature of 21.5–22.0°C.

Results. On account of the preliminary experiments in which the observers were offered collections containing several replicates of each pair, it was found that the standard deviation of the ratio estimates obtained for the weak solutions used as comparison stimuli was the same as that obtained for the stronger ones. If, on the other hand, this deviation is expressed as a fraction (for example, as a percentage) of the mean ratio estimate in question, it is considerably smaller for the stronger solutions which are to be compared This finding does not agree with the results obtained by the ratio-estimation method (see p. 505). This has probably to be interpreted in the light of the methods used. It has to be kept in mind that the observer knew that the standard, i.e. the stronger solution in each pair, was always the last to be presented. If the above-mentioned information had not been available to him, it is probable that he might have considered the solution destined for comparison as stronger than the standard more frequently when assessing the pairs with the stronger solutions than the pairs with the weaker ones. Such misjudgements might have been due, for instance, to a certain variation in the observer's concentration during different phases of the assessments, a possible variation in the way in which the solutions to be compared or the standards had been applied to epithelial surfaces with taste buds, erroneous assessments of possibly occurring irrelevant sensory impressions, etc.

When the estimations of quotients obtained in the experiments described here were plotted for each collection of samples against the concentration of the solutions to be compared, the intensity of sensation and the strength of stimulus seemed to be directly proportional. This seemed to be the case also in the preliminary experiments described above. Because of this, it was convenient to represent this connection by a straight line fitting the points, and to use the equation of this line for the calculation of the concentration $C_{(R/2)}$, which, according to the assessments of the solutions for comparison, should be half of that of the standard, viz. C_R. In this way three or four estimations of $C_{(R/2)}$ were obtained for each standard. The mean value and number of estimations are presented in col. 3 of Table 19. As the strongest standard of quinine hydrochloride was accidentally destroyed before the end of the experiment, only three estimations of $C_{(R/2)}$ for this substance were obtained. During the experiments with phenylthiourea it was not observed that series containing the lowest standard had been subjected to one more test than the others. In the continued treatment of the material the logarithms of the calculated concentrations, $C_{(R/2)}$, were plotted against the logarithmic values of the corresponding C_R. Then a regression line for this connection was calculated according to the method of least squares. In order that the constants and the coefficients of regression may represent the parameters of the data well, the variance of the group of y's associated with any one value of the x has to be the same. For logical reasons we may expect the variances

Table 19. Ratio-calculation data obtained from observer No. 1.

Abbreviations: C_R = conc. (M) of solutions used as standards, $C_{(R/2)}$ = conc. of solutions calculated to produce an intensity of taste half the strength of that of the corresponding standard, n = number of $C_{(R/2)}$ estimated for each standard.

1	2	3	4	5	6	7
			Log $C_R/C_{(R/2)}$			
Compound	C_R	$\bar{X}_{C(R/2)}$	n Regr. line and number of variates	Log C (M)	R	Standardized log scale values
Sucrose	$.175 \times 10^{1}$	.555	4 $y = .7616\,x -$	.2430	100	2.0000
	.877	.349	4 $-.1212\,x^2 -$	− .2497	50	1.6990
	.553	.245	4 $-.4277;$	− .6254	25	1.3979
	.277	.119	4 $n = 28$	− .9514	12.5	1.0969
	.175	$.871 \times 10^{-1}$	4	−1.2620	6.25	.7959
	$.877 \times 10^{-1}$	$.429 \times 10^{-1}$	4	−1.5819	3.125	.4949
	$.553 \times 10^{-1}$	$.268 \times 10^{-1}$	4			
1.3.5-Trinitrobenzene	$.500 \times 10^{-3}$	$.176 \times 10^{-3}$	3 $y = .7817\,x -$	−3.3010	100	1.49
	$.158 \times 10^{-3}$	$.707 \times 10^{-4}$	3 $-1.1807;$	−3.7612	50	1.19
			$n = 6$	−4.1209	25	.88
2.4.6-Trinitrophenol	$.127 \times 10^{-3}$	$.283 \times 10^{-4}$	3 $y = .2516\,x -$	−3.8962	100	1.55
	$.400 \times 10^{-4}$	$.139 \times 10^{-4}$	3 $-.1045\,x^2 -$	−4.5514	50	1.25
	$.127 \times 10^{-4}$	$.612 \times 10^{-5}$	3 $-3.9457; n = 9$	−4.9649	25	.94
Quinine hydrochloride	$.550 \times 10^{-3}$	$.198 \times 10^{-3}$	3 $y = 2.5942\,x +$	−3.2596	100	2.0000
	$.174 \times 10^{-3}$	$.556 \times 10^{-4}$	4 $+.2049\,x^2 +$	−3.6999	50	1.6990
	$.550 \times 10^{-4}$	$.173 \times 10^{-4}$	4 $+2.5796;$	−4.2143	25	1.3979
	$.174 \times 10^{-4}$	$.759 \times 10^{-5}$	4 $n = 15$	−4.7148	12.5	1.0969
				−5.0976	6.25	.7959
Phenylthiourea	$.283 \times 10^{-3}$	$.863 \times 10^{-4}$	3 $y = 1.3968\,x +$	−3.5482	100	1.94
	$.896 \times 10^{-4}$	$.322 \times 10^{-4}$	3 $+.0730\,x^2 -$	−4.0717	50	1.64
	$.283 \times 10^{-4}$	$.134 \times 10^{-4}$	3 $-.0342;$	−4.5119	25	1.34
	$.113 \times 10^{-4}$	$.697 \times 10^{-5}$	4 $n = 13$	−4.8511	12.5	1.03
				−5.0931	6.25	.73

of the $C_{(R/2)}$ obtained for the different standard solutions to be roughly the same, when expressed as fractions of the mean value. In the diagrams obtained (not presented here) the points for sucrose, quinine hydrochloride, phenylthiourea and 2.4.6-trinitrophenol seem to be fitted by a more or less curved line. Thus an equation of the second degree of the type $y = a + bx + cx^2$, where $x = C_R$, $y = C_{(R/2)}$, and a, b, and c are constants, can be expected to fit the points better than a linear equation ($y = a + bx$). Such an equation has in these cases been made to represent the connection between x and y within the range of concentrations examined (Table 19, col. 4). Since the multiple coefficient of correlation for sucrose is close to 1 (not shown here) the points are situated very close to the calculated regression line. The agreement was considered so good that there was no reason for a check with equations of a higher order. The same applies also to quinine hydrochloride, phenylthiourea and 2.4.6-trinitrophenol. The very good fit may partly be explained by the small number of points elaborated. In the course of the preliminary experiments, concentrations of the bitter substances were tasted which were considerably higher than the highest that formed the standard in these experiments. On these occasions the observer

experienced a bitter taste of such intensity that it created nausea, which made the assessment considerably more difficult. At the same time it was extremely difficult to eradicate the taste sensation, even by repeated rinsings. This was the reason why the number of standard solutions examined was limited to some few in these experiments.

For 1.3.5-trinitrobenzene the interval between the absolute threshold and the concentration of saturation is very small. On this account only two standard solutions could be tested. For this reason a linear equation has been made to express the connection between log C_R and log $C_{(R/2)}$ for this substance (col. 4).

In establishing scales for each of the substances, the above-mentioned connection between C_R and $C_{(R/2)}$, represented by the regression lines in question, has been utilized, according to the principles recommended by Ekman (1958) and Björkman & Ekman (1962). The intensity of sensation created by the strongest of the tasted concentrations, i.e. the strongest standard, has been given the arbitrary value 100 (see cols. 5 and 6). The concentration $C^1_{(R\ 2)}$, representing the R value 50, has then been calculated from the equation for the regression line. In the continued scaling the above-mentioned $C^1_{(R/2)}$ was considered as the standard. The $C_{(R/2)}$ corresponding to it has the R value 25 and has been calculated from the equation in the usual way. In the continuation the same method is used for the calculation of the concentrations corresponding to the R values 12.5, 6.25, etc. This is continued until the steps become so small as to be meaningless. All the calculations were carried out on the CD 3600/8090 digital computer (Uppsala Data-processing Centre).

3. *Magnitude-estimation method*

For this purpose solutions of each of the bitter substances 1.3-dinitrobenzene, 1.3.5-trinitrobenzene, 2.4.6-trinitrophenol, and phenylthiourea and of the sweet substances nitrobenzene, 1.2-dinitrobenzene, phenol, resorcinol, phloroglucinol, *o*-nitrophenol, and *p*-nitrophenol were prepared in the way described above (p. 504). The solutions (Table 20 (p. 511), col. 2) were intended to serve as standard stimuli for the taste experiments described in the following pages. In addition series of solutions for comparison were prepared of sucrose and quinine hydrochloride, respectively, the concentration interval being 0.1 log units.

The experiment was carried out along the lines given by Stevens (1956) for the "magnitude-estimation method".

For the experiments a series of six consecutive concentrations of stimuli for comparison were selected for each substance. Preliminary experiments showed that the concentrations in the middle of the series caused roughly equivalent intensities of taste and also, like the other comparison stimuli, had the same quality of taste as the standard in question.

The standard was combined with two samples of each solution for comparison in such a way that the standard was placed as No. 2 in the triad. This was done in order to cancel any possible effects of the order of presentation. All triads containing the same standard were grouped in collections of triads and offered to the observer in random order. Also the sequence in which the different collections of triads were to be tested was determined by means of randomization. The complete experiment was repeated three times.

In the course of the assessment the arbitrary value 100 was given to the intensity of sensation produced by the standard solution. The observer had the task of telling the strength of the different solutions destined for comparison in relation to the

Table 20. Magnitude-estimation data.

Abbreviations: $C_{\text{PSE eqv. standard}}$ = conc. (M) of comparison stimulus, calculated to be equivalent in intensity of taste to that of the standard. The first seven compounds are sweet, while the rest are bitter.

1	2		3	4	5	6
Substance	Conc. (M) of standard	Log conc. (M) of standard	log $C_{\text{P.S.E. eqv. standard}}$ of comparison stimuli in collection of triads No. 1	2	3	$\bar{X}_{\text{log PSE. eqv.standard}}$
Nitrobenzene	$1.74 \cdot 10^{-3}$	−2.7595	−1.0023	− .9343	− .8992	− .9453
1.2-Dinitrobenzene	$7.20 \cdot 10^{-4}$	−3.1415	− .9544	− .9616	− .7351	− .8837
Phenol	$3.21 \cdot 10^{-2}$	−1.4935	−1.1321	− .9482	−1.1701	−1.0835
Resorcinol	$2.71 \cdot 10^{-2}$	−1.5670	−1.1533	−1.0584	−1.0655	−1.0924
Phloroglucinol	$1.03 \cdot 10^{-2}$	−1.9872	−1.2503	−1.2099	−1.2171	−1.2258
o-Nitrophenol	$7.55 \cdot 10^{-3}$	−2.1221	−1.1574	−1.0395	−1.0597	−1.0855
p-Nitrophenol	$1.64 \cdot 10^{-2}$	−1.7852	−1.2789	−1.2293	−1.2093	−1.2391
1.3-Dinitrobenzene	$1.24 \cdot 10^{-3}$	−2.9080	−4.5218	−4.5688	−4.4007	−4.4971
1.3.5-Trinitrobenzene	$2.84 \cdot 10^{-4}$	−3.5467	−4.3936	−4.2989	−4.3048	−4.3324
2.4.6-Trinitrophenol	$4.00 \cdot 10^{-5}$	−4.3979	−4.3865	−4.3231	−4.3287	−4.3461
Phenylthiourea	$8.96 \cdot 10^{-5}$	−4.0477	−3.7861	−3.6973	−3.8370	−3.7735

standard. If its strength was considered to be 75 %, the figure 75 was to be given, if it was considered to be 25 % stronger, this was to be expressed by the figure 125, etc. Since the standard solution and the solution for comparison had been made up of different substances, the mouth had to be rinsed after taking the individual preparations in each triad. Otherwise the presence of one of the substances might conceivably have interfered with the possibility of the other substance's acting upon the excitable system in the receptors. Investigations aimed at the study of phenomena of this kind may be of interest in connection with future work, but are not topical at the present moment.

In the assessment of each triad the first preparation was tasted for 6 seconds and then discharged. During the following 6 seconds the mouth was repeatedly rinsed with "distilled" water drawn from a siphon. This was connected by means of a polyvinyl-chloride tube with a bottle containing previously prepared "distilled" water of suitable pH and suitable temperature, and could easily be manipulated by the observer. Afterwards the standard solution was tasted for 6 seconds. During this time the observer carried out an assessment keeping the figure in mind. After the discharge followed renewed rinsings, according to the method just described. Now the second sample of the solution for comparison was introduced into the mouth and kept there for 6 seconds. It was assessed against the memory of the strength of the standard solution. Upon the basis of these two estimations the observer subsequently gave a final scale value. Otherwise these experiments were carried out according to the procedures used previously with the other psychophysical methods. The temperature of the samples before tasting was 22.1°C.

Results. From the results obtained previously for the scaling of quinine hydrochloride and sucrose, it seems to be adequate to represent the connection between the log values of the intensity of the sensation and of the strength of the stimulus

by a straight line, at least within the relatively narrow range of concentrations covered by the solutions for comparison in each collection of triads.

Thus, a straight line representing the regression of the logarithm of the scale value (y) upon the logarithm of the concentration (x) has been calculated for each collection of triads by a digital computer. Then x has been calculated for $y = \log 100$ (Table 20, cols. 3–5). From the estimations obtained, this concentration can be expected to cause the same intensity of taste sensation as the standard solution and is thus an estimation of log P.S.E. The arithmetical mean is given in col. 6. As can be seen, there is a considerable variation for all substances.

Discussion

The scale values obtained for sucrose and quinine hydrochloride by means of the ratio-calculation method were entered on the log-log diagram (Fig. 5). After uniting adjacent points by straight lines, the mean value of the P.S.E. for each of the remaining substances was introduced by interpolation. Then the scale values obtained for each substance by the ratio-estimation or ratio-calculation method were inserted in the diagram and connected by lines, The graph obtained was then shifted upwards or downwards until it or its extension intersected the mean value of the P.S.E. for the compound in question. The new scale values, which may be immediately read off on the ordinate in the diagram, will be found in Table 18 (p. 506), col. 12, and Table 19 (p. 509), col. 7 for the different compounds tested.

The diagram shows that the lines representing the connection between R and S and obtained by the ratio-estimation method exhibit a flatter slope than those obtained by the ratio-calculation method. It is impossible to tell from the few data underlying the scales whether the difference is due only to the application of different methods or to a change with time in the constants which determine the connection between the intensity of sensation and the strength of the stimulus in the observer. The ratio-calculation method was put to the test a couple of days after the termination of the experiments, using the ratio-estimation method.

The types of function obtained for sucrose and quinine hydrochloride with the ratio-calculation method by Lewis and the present author seem to agree, as they are approximately linear. However, the functions obtained by Lewis show a markedly steeper slope. Furthermore these lines seem to have a straighter course. Whether and to what extent this can be explained by difference in the methods involved cannot yet be evaluated. The observers directed by Lewis had to select a stimulus that was judged to be half as strong as the standard presented from a series of comparison stimuli arranged in descending order of concentration. They were, however, cautioned about the tendency to choose the middle range stimuli during the test but were also warned not to over-compensate for this effect.

In a communication on an electrophysiological investigation on human beings Diamant *et al.* (1963) have reported on the connection between the strength of the stimulus and the integrated response in the chorda tympani, for example, when sucrose was used as stimulus. A look at Fig. 3 in their paper shows the curve in question to be upwardly concave. If the most exact scale values for sucrose, obtained by the ratio-calculation method, are introduced into a similar diagram, a curve with essentially the same course is obtained. In Fig. 6 the response units are equated by letting the two curves pass through the same point for a 0.5 M solution. The similar course seems to point to a simple connection between the intensity of the sensation

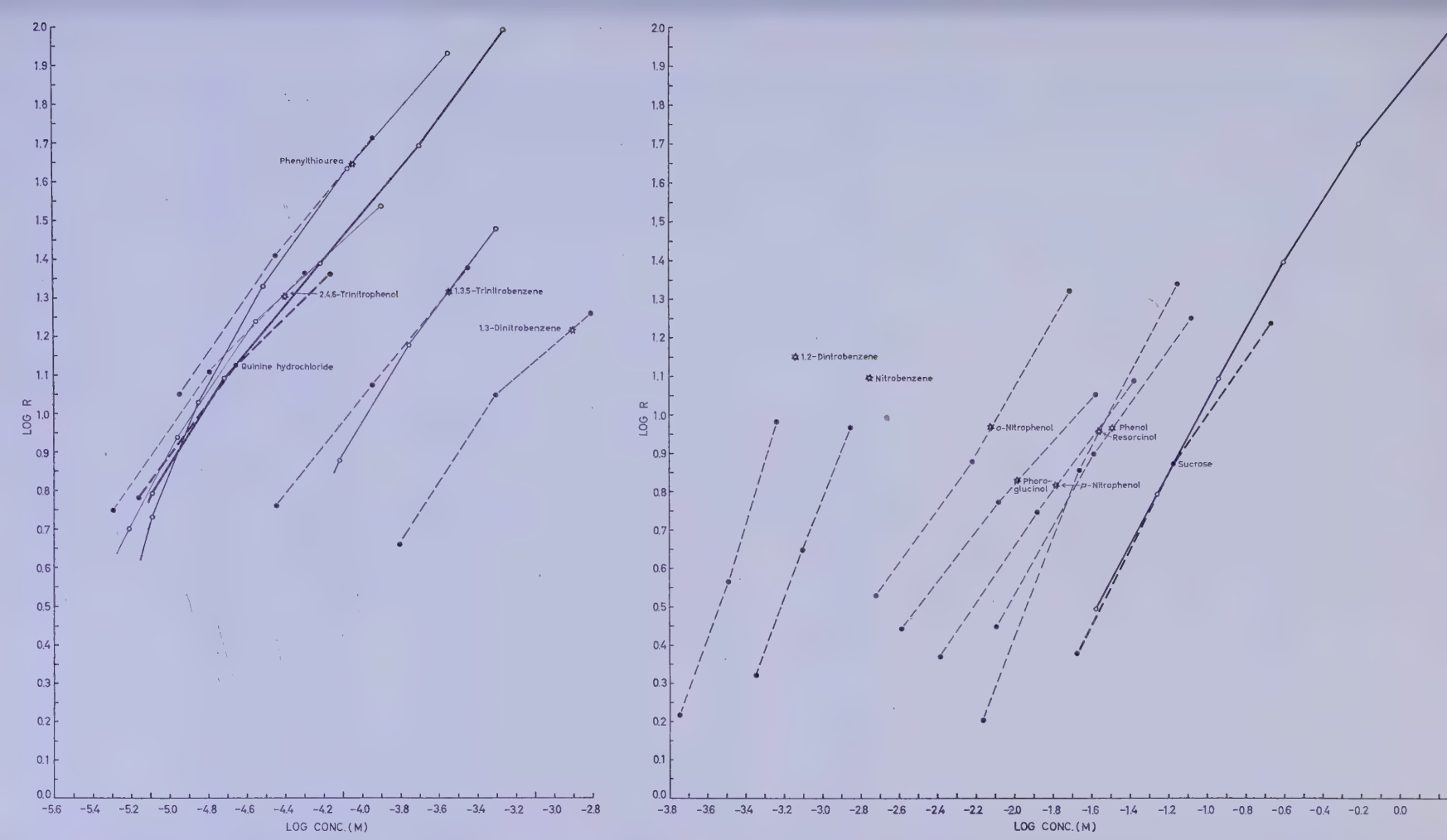

Fig. 5. The intensity of sensation as a function of the strength of the stimulus for the compounds tested. All values of R for sweet and bitter compounds are expressed in t same units as those for sucrose and quinine hydrochloride, respectively. The continuous and broken lines represent data obtained according to the ratio-calculation and rat estimation method, respectively. Symbols ★ show points of subjective equality, obtained by the magnitude-estimation method.

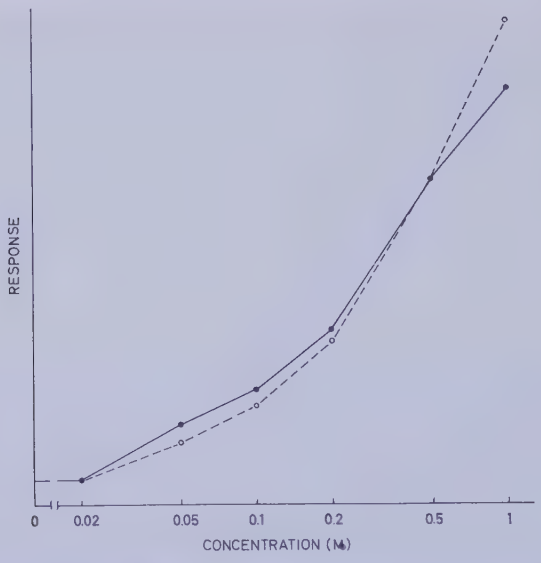

Fig. 6. Graph showing (*a*) the integrated response in the chorda tympani and (*b*) the intensity of sensation as a function of the logarithm of the molarity of the sucrose solution (continuous and broken line, respectively). Data according to Diamant *et al.* (1963) and the present author.

and the frequency of discharge in the taste nerve. Similar conclusions seem to be logical in a comparison of the response-stimulus functions obtained in electrophysiological experiments by Diamant *et al.* (1963) and in psychophysical experiments with sodium chloride performed in this laboratory (Marcström 1965) with the same technique as that in the present work. Conclusive evidence can, of course, be obtained only if both the psychophysical and the electrophysiological experiments are carried out with the same observer. Such experiments have in fact been performed with citric acid at Zotterman's laboratories in Stockholm (Zotterman 1966). They show a linear relation between the intensity of sensation and the frequency of discharge in the chorda tympani. The same simple connection is valid as regards sucrose and sodium chloride according to Borg *et al.* (in press).

Provided that there really is a direct connection between the intensity of sensation and the frequency of discharge of nerve impulses in the taste nerves and furthermore between the latter and the intensity of the initial reaction between the stimulus and the excitable system in the receptors, the relationship observed between R and S may, according to Beidler's taste equation (see p. 429), picture these adsorption processes.

Under these conditions a plot of C/R against C in a diagram should give a straight line, if the stimulating molecules are bound by a set of equivalent, independent sites in a monomolecular reaction. In fact, such a straight line appears, for example, when the data obtained by the ratio-calculation method for sucrose are treated according to Beidler (1954).

As, on the other hand, the points for some of the compounds tested seem to be well fitted by a straight line in the log-log plot in Fig. 5, the R-S relationship in these cases can undoubtedly be successfully expressed by power functions. Such a function

is the Freundlich equation $x/m = k \times C^n$, which, according to Daniels and Alberty (1961), sometimes describes the data obtained in adsorption experiments, at least for low coverages, better than the Langmuir isotherm. In this equation x is the amount of substance adsorbed on the mass m of the adsorbent, C is the concentration, and k and n are constants. The latter can be determined by using a log-log plot, since $\log x/m = \log k + n \log C$; n is thus the slope of the line and k is the antilogarithm of the intercept. The equation is derived, assuming a heterogeneous surface, with the adsorption on each class of sites obeying the Langmuir equation. Provided, as is earlier assumed, that there is a direct connection between the intensity of sensation and the intensity of the initial reaction between the stimulus and the excitable system in the receptors, there is thus a possibility that the intensity of the initial reaction is in turn directly proportional to the amount of stimulus adsorbed on the receptors in question.

The following conclusion may be drawn from the application of the data for the compounds tested in the present research to the two equations mentioned above. For some of the substances the data seem to fit either theorem quite well, whereas for others the data diverge slightly from both of them. In general also the data obtained by the ratio-estimation method fit one of the equations, whereas the data obtained for the same compound by the ratio-calculation method fit the other equation better. The fit of the data to these equations seems to favour the idea that the process of stimulation may be based upon adsorption processes. The conclusive power of this idea is, however, reduced by the observations made by several authors that the equations in question also fit data obtained in a similar way for other sense modalities (cf. Duncan (1963), Stevens (1960)).

It has already been mentioned (p. 426) that an increase, by a certain factor, of the strength of stimuli affecting the excitable system in the receptors in the same way may be expected to result in an equal increase in the intensity of the sensation. Thus, the curves representing the connection between log R and log S for such substances may be expected to show approximately the same slope. Obviously, however, the curves obtained for some of the substances by the ratio-estimation method (Fig. 5) differ in this respect remarkably from those representing the rest of the compounds, the differences being large enough to prevent their being explained by chance. Thus, the slope of the curves representing the sweet substances nitrobenzene, 1.2-dinitrobenzene, and resorcinol agree with each other, but deviate significantly from the rest. As all the experiments were performed under very similar conditions (p. 505), this may indicate that the two different groups of substances affect the excitable system(s) in the receptors in different ways. It appears furthermore that the line representing the scale values for phenylthiourea has a fairly steeper slope in the lower region of concentrations tested than the lines for other bitter compounds tested, among them especially 1.3-dinitrobenzene.

IX. Stimulating potency and number of moles per unit of volume in saturated aqueous solutions of the compounds tested

Most authors concerned with the problem of how the excitable system in the taste receptors is brought into action have probably looked upon the solubility of the stimulating substance in water only as a property necessary for the transport of the molecules in question to the receptors. Only in a few cases has the solutbility of

compounds in water been mentioned in discussions concerning their stimulating ability. Taylor (1928) pointed out that two of the sweetest tasting substances known, α-anti-aldoxime of perillaldehyde and *n*-hexyl chlormalonamide, have very low solubilities in water and thus cannot be brought into contact with the "taste system" in high concentration. The intensely sweet sacharine is again only slightly soluble in water. Loginov proposed in 1941 that the solubility of a sugar in water divided by the solubility of sucrose in water should be used as an index of sweetness. Andersen *et al.* (1963), after examining the stimulating abilities of some mono- and disaccharides on mongrel dogs, using an electrophysiological technique, stated that greater solubility in water, expressed as a percentage by weight, can be associated with stronger stimulating ability. This relation, however, seems to hold only within each group of compounds mentioned when the solubility is expressed in moles.

Determination of the solubility in water of some of the compounds tested

I considered it also to be of interest to study the relationship between the stimulating ability and the solubility of compounds in water. The latter quantity is here represented by the number of moles present per unit of volume in saturated aqueous solutions.

The literature contains few data concerning the solubility at different temperatures of the compounds, used in the present investigation. There seem to be no data whatever for different pHs. However, for other reasons not reported here, I made measurements of this characteristic in some of the substances under conditions (pH 5–6, 22°C) similar to those prevailing in the sensory areas in some of the taste experiments performed (pH about 5.9, 27°C). These data have been used for a preliminary study of the connections between the above-mentioned factors (solubility and the stimulating ability of the compounds).

Method

A sufficient amount of an actual substance was transferred to a glass bottle containing distilled water boiled in advance for 15 minutes to remove the gases (O_2, N_2, CO_2) present. After the air over the liquid surface had been replaced by N_2 (for the prevention of oxidation of hydroquinone, pyrogallol and phloroglucinol, compare p. 440), the bottles were sealed and shaken in a water bath at 22.0°C (standard deviation 0.1°C) for 12 hours. The solutions were then titrated (glass electrode, type G 200*b* and calomel electrode, type K 100) to the desired values of pH between 5 and 6 by adding suitable amounts of NaOH or HCl. The shaking was then continued for another 12 hours, after which the preparations were left in the water bath overnight. Three independent measurements of the concentration (expressed in moles) of each compound were made, after suitable dilution, with the aid of a Beckman spectrophotometer (Model DU) at a peak of the absorption curve of the substance in question or, if the compound was protolyzed, at a suitable isosbestic point (p. 441, Table 1 (p. 437), col. 14).

Results

The mean value of the data obtained are given in Table 21 (p. 518), col. 4, together with the pH at the determinations (col. 2). For comparison, I also present the data found in the literature for the solubility of the compounds in water at temperatures in the vicinity of 22°C (cols. 6 and 7). The molality (col. 8) and, in cases in which the specific

density is presented, the molarity (col. 9) of the saturated solutions are also calculated. In those cases in which the substance is infinitely soluble in water, I have calculated the number of moles which constitute one litre. From Table 21 it appears that the differences between my values for the solubilities and those found in the literature are especially large in the cases of *m*-nitrophenol, benzaldehyde and 2.6-dinitropenol, the higher value being respectively *c*. 2.0, 2.8 and 4.6 times greater than the lower one.

Discussion of the results

The logarithms of the concentrations of each substance, representing the mean threshold values of subject No. 1 (Table 21, col. 10*b*) and No. 2 (col. 10*c*), obtained at pH 5.9 and 27°C, have been plotted in a diagram (Fig. 7, p. 520) against the corresponding logarithms for the concentrations of saturated solutions of the compounds in question. These values are printed in italic type in each of the columns 4, 8 or 9 in Table 21 and presented in logarithmic form in col. 10*a*. The threshold values found in Group II (col. *d*) at the lower pH level tested have been treated in the same way in Fig. 8 (p. 521). Only the first estimated mean threshold value of resorcinol is introduced into the diagram.

As may be seen from Figs. 7 and 8, no connection between stimulating ability and solubility in water appears when *all* sweet or bitter compounds are considered. An almost direct proportionality between these characteristics seems to exist, however, regarding the compounds which, according to the observers in group I, have only a bitter taste (Fig. 7). It may thus be profitable to search for this kind of relationship within smaller groups of substances, the members of which also affect the taste apparatus in a similar way under different experimental conditions. In the general discussion below, the available material is treated in this way.

General discussion

The substances have been divided into groups, according to (*a*) their taste quality, (*b*) the stimulated area of the tongue, (*c*) the results obtained from the variance analyses of the influence of pH and temperature on the threshold values, and (*d*) the relationship between stimulating ability and intensity of sensation. A survey of these empirical characteristics of the compounds is presented in Table 22 (p. 522).

§ 1. The compounds concerned are 1.3- and 1.4-dinitrobenzene, 1.3.5-trinitrobenzene, 2.4.6-trinitrophenol, quinine hydrochloride and phenylthiourea and are shown in Figs. 7 and 8. The points in Fig. 7 for these substances, except that for 1.3-dinitrobenzene, seem to fall on a line which curves slightly upwards. It has a slope approaching a value of -1 in the neighbourhood of the point where it crosses the line fitting the points for hypothetical compounds, the thresholds for which are equal to the solubility in water (the diagonal drawn in the graph). All the substances give a bitter taste but react, according to the variance analysis, in different ways with the excitable system of the receptors when pH and temperature are changed (Table 22, col. 3). There is a trend towards a steeper slope for the *R-S* function of phenylthiourea found by the ratio-estimation method and a less steep slope for 1.3-dinitrobenzene, compared with the functions of the other bitter compounds tested (cf. Fig. 5 (p. 513) and Table 22, col. 5), a circumstance which probably indicates differences in the way in which the compounds react with the excitable system.

The points in Fig. 8 for the actual substances are distributed in similar way to that in Fig. 7. Here they seem to fall on an approximately straight line with a slope greater

Table 21. Solubility in water of compounds tested.

Superior figures refer to the temperatures in degrees C, r = room temperature and (?) = temperature not stated. Subscripts in col. 10*d* at a given mean threshold value refer to the number of observers in a subgroup within group II (20 observers).

⚹ = Hodgman, Ch. D., Weast, R. C. and Selby, S. M., *Handbook of Chemistry and Physics*, Chem. Rubber Publishing Co., Cleveland, 1961.
△ = Seidell, A., *Solubility of Organic Compounds*, I, II, D. Van Nostrand, New York, 1941.
· = Washburn, E. W., *International Critical Tables of Numerical Data, Physics, Chemistry and Technology*, National Research Council of the U.S.A., McGraw–Hill, New York, 1926.

Meaning of abbreviations in cols. 10*b*, *c* and *d* are explained in Tables 5 and 14.

1		2	3	4	5	6	7	8	9	10							
										a	b	c				d	
		Solubility according to the author's determination			Solubility according to literature					Log Molarity or molality (m) of saturated aqueous solutions	Log mean threshold value of						
											Observer No. 1			Observer No. 2		Group II	
Substance tested																	
Mol. Wt.	Name	pH	g/1000 ml solution	Molarity of saturated solution, × 10^4	Lit. reference	g/1000 g H_2O	g/1000 g solution	Molality of saturated solution, × 10^4	Molarity of saturated or concentrated solution, × 10^4		Dist. w. 27°C, × 10^6M	Threshold named	dist. w. 27°C, × 10^6 M	Threshold named	Lower pH level, × 10^6 M	Threshold named
78.11	Benzene				⚹	1.75^{20}		224								
					△	1.80^{25}		230								
123.11	Nitrobenzene	5.50	2.08	*169*	⚹	1.9^{20}		154		−1.772	−3.760	Sw.	−3.775	Sw.	−3.409	Sw.
168.11	1.2-Dinitrobenzene	5.50	0.133	*7.91*	△	$.14^{r}$		8.33		−3.102	−4.142	Sw.	−4.111	Sw.	−3.757	Sw.
168.11	1.3-Dinitrobenzene	5.44	0.577	*34.3*	⚹	$.525^{r}$		31.2		−2.465	−4.209	Bi.	−4.163	Bi.	−3.991	Bi.
168.11	1.4-Dinitrobenzene	5.49	0.0826	*4.91*	△	$.08^{r}$		4.76		−3.309	−4.206	Bi.	−4.291	Bi.	−3.951	Bi.
213.11	1.3.5-Trinitrobenzene	5.45	0.384	*18.0*	△	$.278^{15}$		13		−2.745	−4.848	Bi.	−4.921	Bi.	−4.496	Bi.
					⚹	$.4^{16}$		18.8								
94.11	Phenol	5.46	86	*9,140*	△	91.2^{20}			8,900	− .039	−2.494	Sw.	−2.478	Sw.	−2.214	Sw.
					△	94.8^{25}			9,240							
110.11	Pyrocatechol	5.35	378	*34,300*	⚹	451^{20}		41,000		.535	−1.917	An.	−1.917	An.		
110.11	Resorcinol				△	$1,230^{20}$			*56,800*	.754	−2.567	Sw.	−2.542	Sw.	−2.341	Sw.
					△	$1,430^{25}$			71,400							
110.11	Hydroquinone	5.43	64.5	*5,860*	△		67^{20}	6,520		− .232	−1.780	Sw.	−1.686	Sw.	−1.807	Sw.
					△				6,600							
126.11	Pyrogallol				⚹△	625^{25}		*49,600*		$.696^{m}$	−1.893	Bi.	−1.983	Bi.	−1.767	Bi.
															−1.896	Sw.t.r.
126.11	Phloroglucinol	5.35	14.8	*1,170*	⚹△	11.3^{20-25}		896		− .932	−2.987	Sw.	−3.053	Sw.	−2.845	Sw.
139.11	*o*-Nitrophenol	5.56	1.83	*132*	⚹	2.1^{20}		151		−1.879	−3.122	Sw.	−3.259	Sw.	−2.987	Sw.

139.11	*m*-Nitrophenol	5.50	10.7	*769*	△		21.4[20]	1,570		−1.114	−3.654	Sw.	−3.575	Sw.	−3.287	Sw.
					⚹	13.5[25]		970								
139.11	*p*-Nitrophenol	5.54	12.2	*877*	△		13.2[20]	962		−1.057	−2.785	Sw.	−2.717	Sw.	−2.502	Sw.
					⚹	16[25]		1,150								
					·				1,097							
184.11	2.4-Dinitrophenol	5.70	1.86	*101*						−1.996	−3.097	Sw., Bi.	−3.208	Sw., Bi.	−2.903	Sw.
184.11	2.5-Dinitrophenol	5.53	13.5	*733*						−1.135	−2.562	Sw.	−2.562	Sw.	−2.804	Sw.
184.11	2.6-Dinitrophenol	5.67	1.44	*78.2*	△	.315[15]		17.1		−2.107	−3.077	M.	−3.582	M.		
229.11	2.4.6-Trinitrophenol	5.40	12.4	*541*	⚹	14[20]		611		−1.267	−5.697	Bi.	−5.662	Bi.	−5.444	Bi.
					·				550							
106.12	Benzaldehyde	5.68	8.53	804	△	3[r]		283								
					·	4[25]		377								
152.14	Vanillin	5.46	8.44	555	△	10[20–25]		657								
138.6	Veratrole	5.76	6.92	499	△											
342.30	Sucrose				△		670.9[20]		*26,050*	.416	−2.081	Sw.	−1.910	Sw.	−1.804	Sw.
					△		678.9[25]		26,580							
396.91	Quinine hydrochloride	5.90	44.0	*1 100*	△		32.07[18]	834.8		− .959	−5.561	Bi.	−5.703	Bi.	−5.396	Bi.
					△		49.11[25]	1,301								
152.21	Phenylthiourea	5.85	2.37	*156*	△		2.12[20]	140		−1.807	−5.349	Bi.	− 5.417	Bi.	-4.799_{18}	Bi.
					△		2.4[25]	158							-3.166_{2}	Bi.
69.1	Sodium nitrite				△		450.7[22]		*88,170*	.945	−2.648	Sw.			−2.520	M.
															-2.516_{19}	Sw.
85.10	Potassium nitrite				△		743[20]		*144,000*	1.158					−2.516	M.
															-2.506_{18}	Sw.
61.04	Nitromethane				△		100 g/1000 ml[25] solution		*14,900*	.173					− .799	Sw.
75.07	Nitroethane				⚹	45 ml/1000 ml (?) H_2O		*6,310*		− .200[m]					−1.336	Sw.
89.09	1-Nitropropane				⚹	14 ml/1000 ml (?) H_2O		*1,580*		− .801[m]					−1.975	M.
															−1.728	Sw.
89.09	2-Nitropropane				⚹	17 ml/1000 ml (?) H_2O		*1,890*		− .724[m]					−1.686	M.
															-1.760_{14}	Bi.
32.04	Methanol				⚹	∞			*247,400*	1.393					.072	M.
															.367	Sw.
															.225	Bi.
46.07	Ethanol				⚹	∞			*171,300*	1.234					− .068	M.
															.362	Sw.
															.009	Bi.
60.09	2-Propanol				⚹	∞			*130,700*	1.116					− .387	M.
															$-.313_{19}$	Bi.
															$.053_{14}$	Sw.

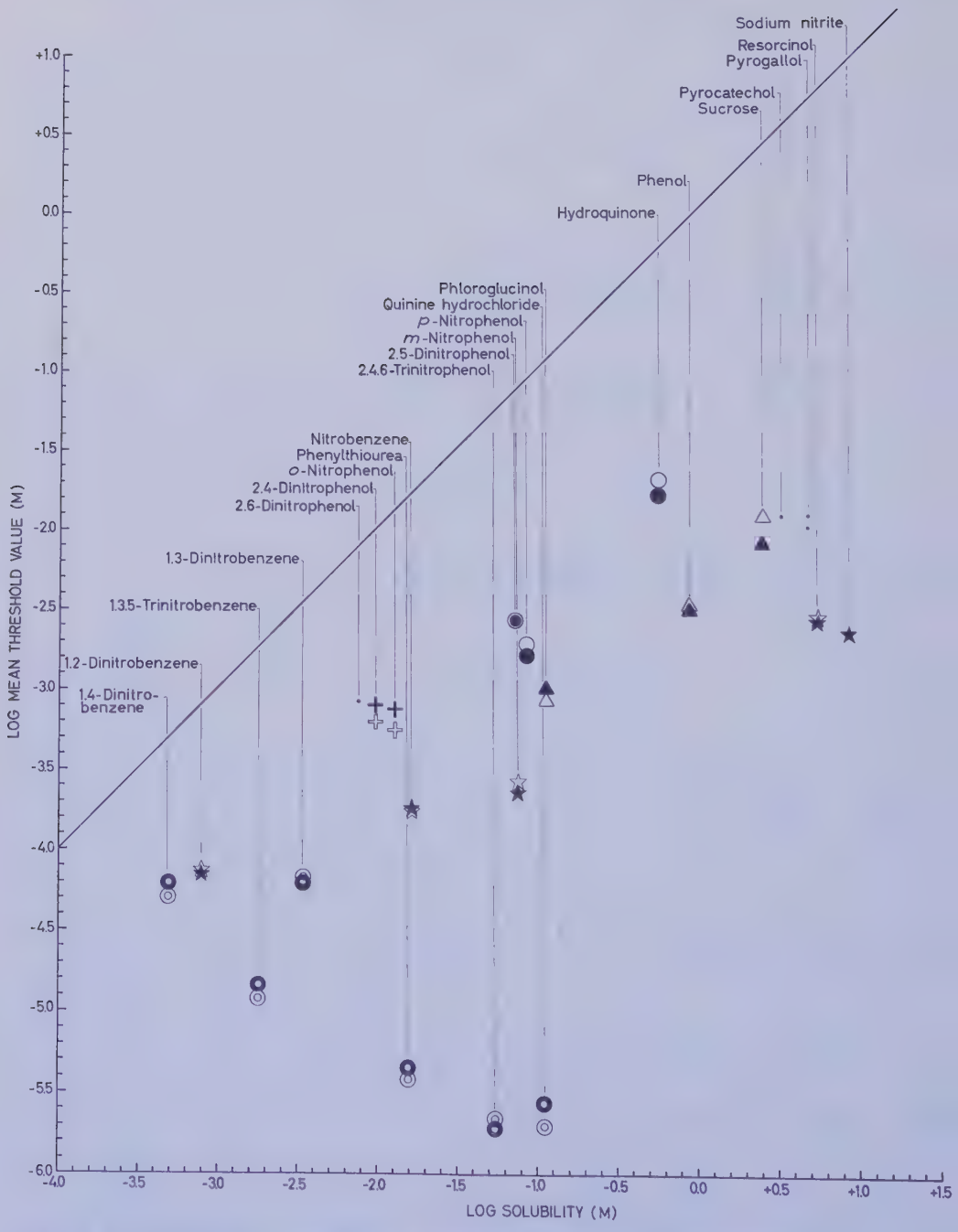

Fig. 7. Logarithm of the mean threshold value of observer No. 1 (black symbols) and No. 2 (open symbols) (Table 21, cols. 10*b* and *c*) as a function of the logarithm of the number of moles per unit of volume in saturated aqueous solutions of the compounds tested (Table 21, col. 10*a*). Meaning of symbols used

○ = compound discussed under § 1 (assigned to group 1).
★ = compound discussed under § 2 (assigned to group 2).
▲ = compound discussed under § 3*a* (assigned to group 3).
● = compound discussed under § 3*b* (assigned to group 4).
\+ = compound discussed under § 4 (assigned to group 5).
. = compound not assigned to any group.

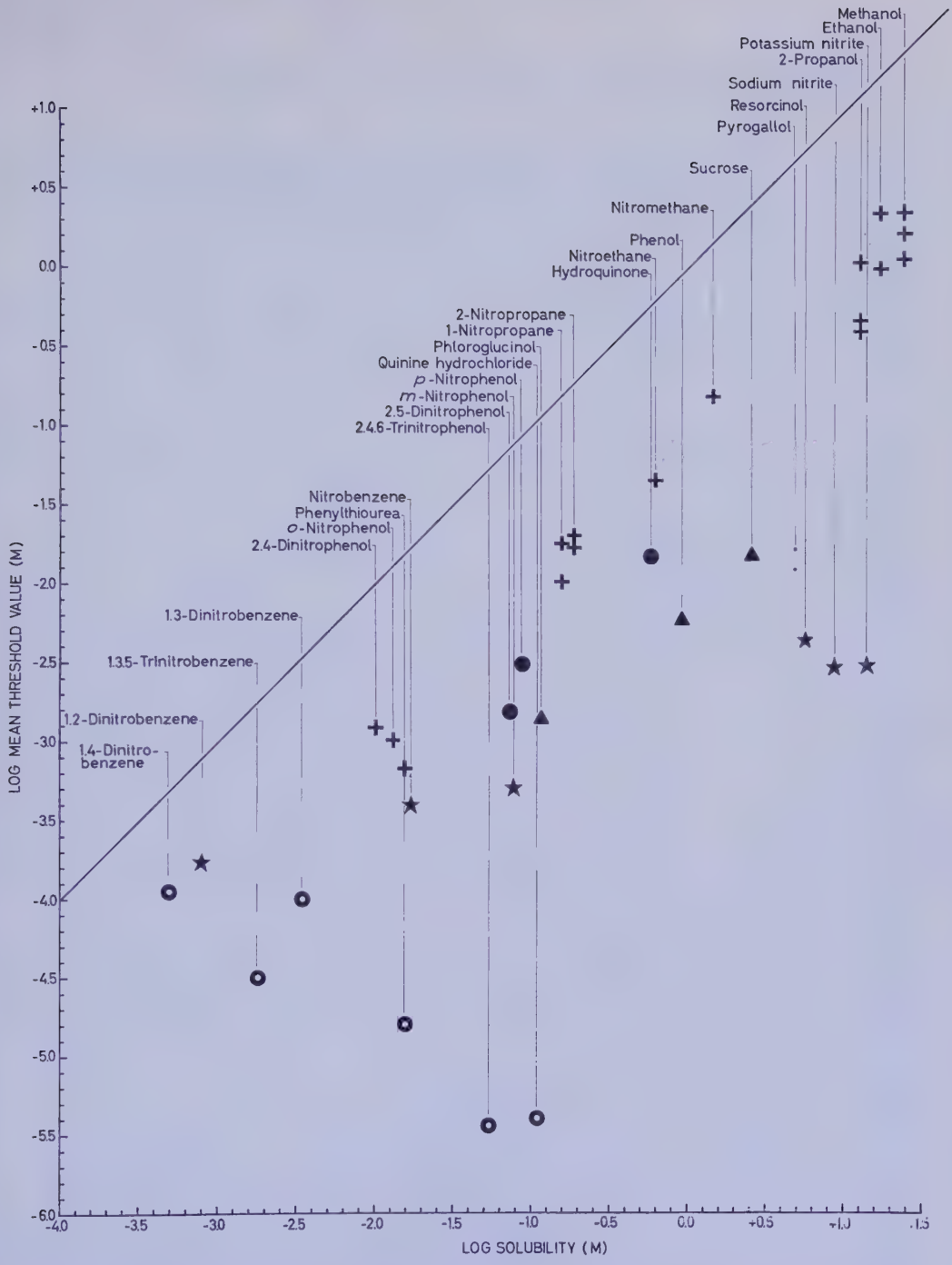

Fig. 8. Logarithm of the mean threshold value of group II as a whole (Table 21, col. 10*d*) as a function of the logarithm of the number of moles per unit of volume in saturated aqueous solutions of the compounds tested (Table 21, col. 10*a*).
Meaning of symbols used

○ = compound discussed under § 1 (assigned to group 1).
★ = compound discussed under § 2 (assigned to group 2).
▲ = compound discussed under § 3*a* (assigned to group 3).
● = compound discussed under § 3*b* (assigned to group 4).
\+ = compound discussed under § 4 (assigned to group 5).
. = compound not assigned to any group.

Table 22. Survey of empirical characteristics—(1) taste quality, (2) stimulated area of the tongue, (3) results of the variance analyses of the influence of observer, temperature and quality of the water on the size of the threshold value and (4) the relationship between intensity of sensation and strength of stimulus—used as arguments for the distribution of compounds in Figs. 7 and 8.

Kinds of sensations reported by 5 or more of the 20 observers in group II are included for each compound in order in col. 2*c*. Sensations within parentheses were noticed by less than 10 observers. The abbreviations in col. 2 and in the heading of col. 3 are those used in Tables 14 and 15 respectively. A question mark indicates that the characteristic shown in the actual col. has not been empirically examined.

1		2			3				4	5
a	b	a	b	c	a	b	c	d		
Compound		Sensations evoked according to:			Variance analyses (Group I) Mean threshold value lower at lower (L) or higher (H) level of factor			Evidence (+) and no evidence (0) of interaction W × T	*t*-test (Group II) Mean threshold value lower at lower (L) or higher (H) level of pH	Slope of log *R*-log *S* function steeper (>) and less steep (<) than the one most frequently observed (medium)
Found under paragraph	Name	Observer No. 1	Observer No. 2	Group II	O	W	T			
1	1.3-Dinitrobenzene	Bi.	Bi.	Bi.				0	L	<Medium
	1.4-Dinitrobenzene	Bi.	Bi.	Bi.	H	L	H	0	L	(?)
	1.3.5-Trinitrobenzene	Bi.	Bi.	Bi.	H			+		Medium
	2.4.6-Trinitrophenol	Bi.	Bi.	Bi.		L		0	L	Medium
	Quinine hydrochloride	Bi.	Bi.	Bi.	H	L		+	(?)	Medium
	Phenylthiourea (tasters)	Bi.	Bi.	Bi.	H		H	+	(?)	>Medium
2	Nitrobenzene	Sw.	Sw.	Sw.		L	H	+		>Medium
	1.2-Dinitrobenzene	Sw.	Sw.	Sw.		L		0	L	>Medium
	Resorcinol	Sw.	Sw.	Sw.		L		+		>Medium
	m-Nitrophenol	Sw.	Sw.	Sw.	L	L	H	+		(?)
	Sodium nitrite	Sw., Sal., Metal	Sw., Metal	Sw., (Sal.)	(?)	(?)	(?)	(?)	(?)	See p. 507
	Potassium nitrite	Sw.	Sw., Metal	Sw., (Sal.)	(?)	(?)	(?)	(?)	(?)	(?)
3	Sucrose	Sw.	Sw.	Sw.	L		H	0		Medium
	Phenol	Sw., An.	Sw., An.	Sw., An.		L	H	0		Medium
	Phloroglucinol	Sw.	Sw.	Sw.		L		0		Medium
	Hydroquinone	Sw., Sal., As.	Sw., Sal., As.	Sw., (Bi.)	L	H		0		(?)
	p-Nitrophenol	Sw., Bu.	Sw., As.	Sw.	L	H		0	(H)	Medium
	2.5-Dinitrophenol	Sw., Bu.	Sw., Bu.	Sw., Bu.	(?)	(?)	(?)	(?)		(?)
4	*o*-Nitrophenol	Sw., Bi.	Sw.	Sw.	H	L		0		Medium
	2.4-Dinitrophenol	Sw., Bi., Bu.	Sw., Bi.	Sw., Bi.	H			0		(?)
	Nitromethane	Sw., Bi.	Sw., Bi.	Sw., Bi.	(?)	(?)	(?)	(?)	(?)	(?)
	Nitroethane	Sw., Bi.	Sw., Bi.	Sw.	(?)	(?)	(?)	(?)	(?)	(?)
	1-Nitropropane	Sw., Bi.	Sw., Bi.	Sw., Bi.	(?)	(?)	(?)	(?)	(?)	(?)
	2-Nitropropane	Bi.	Bi.	Bi.	(?)	(?)	(?)	(?)	(?)	(?)
	Methanol	Sw., Bi.	Sw., Bi.	Sw., Bi.	(?)	(?)	(?)	(?)	(?)	(?)
	Ethanol	Sw., Bi.	Sw., Bi.	Sw., Bi.	(?)	(?)	(?)	(?)	(?)	(?)
	2-Propanol	Sw., Bi.	Sw., Bi.	Sw., Bi.	(?)	(?)	(?)	(?)	(?)	(?)
	Phenylthiourea (non-tasters)			See p. 488	(?)	(?)	(?)	(?)	(?)	(?)

than −1. It should be noted that the point representing phenylthiourea is the mean threshold value of the subgroup of 18 tasters.

1.3-Dinitrobenzene markedly deviates from the line representing the group. The preparations of this compound used for the estimation of the threshold values and the solubility were as will be seen from Table 1 (p. 436), of lower purity than those of the other bitter compounds studied. This should not have induced such a large deviation, provided that the impurities did not in some way interfere with the reaction between the excitable system and the stimulating molecules in question. There is also a possibility that 1.3-dinitrobenzene belongs to another group of substances which is not otherwise represented.

§ 2. The compounds concerned are nitrobenzene, 1.2-dinitrobenzene, resorcinol, *m*-nitrophenol, sodium nitrite and potassium nitrite.

The points in Fig. 7 representing the first five (subject No. 1) or four (subject No. 2) substances mentioned fall on a slightly upward-curving line. In the variance analyses performed on sweet compounds I found evidence of the W × T interaction only for nitrobenzene, *m*-nitrophenol and resorcinol (Table 22, col. 3*d*). Obviously these substances also appear together in the vicinity of 1.2-dinitrobenzene, when ranked according to effect of the factors O and W (Table 16 (pp. 496–497)). Further the *R*-*S* functions of nitrobenzene 1.2-dinitrobenzene and resorcinol have a steeper slope than the other tested substances (Table 22, col. 5). *m*-Nitrophenol was not tested in this respect. All the compounds give a sweet taste near the threshold. In higher concentration, however, sodium nitrite and potassium nitrite also evoke the sensation of "metal" (Table 22, col. 2, Table 14 and p. 489), while, according to observer No. 1 (p. 507), at the same time the sweet taste disappears. Obviously further experiments will have to be performed, aiming at discovering whether or not they appear merely by chance in the vicinity of the curve fitting the other compounds mentioned.

A straight line seems to fit the points in question in Fig. 8 fairly well. The mean threshold value of group II as regards the electrolytes is mixed (cf. p. 482), although the sweet taste in most cases seems to be predominant in the vicinity of the threshold.

§ 4. The compounds concerned are *o*-nitrophenol, 2.4-dinitrophenol, nitromethane, nitroethane, 1- and 2-nitropropane, methanol, ethanol, 2-propanol and finally phenylthiourea, when presented to non-tasters. According to Table 14 (p. 475 ff.), p. 485 ff., and Table 22, col. 2, the substances (except possibily 2-nitropropane) seem to be able to evoke both a sweet and a bitter taste at the same time. The sweet-and-bitter compounds include, first of all, methanol and ethanol but also 2-propanol, nitromethane, 1-nitropropane and 2.4-dinitrophenol, according to the judgements of observers in group II. Nitroethane and also, according to subject No. 1 in group I, *o*-nitrophenol show a bitter taste in higher concentrations than the threshold value. Only *o*-nitrophenol and 2.4-dinitrophenol have been thoroughly tested by group I. It appeared that observer No. 2 had a significantly higher sensitivity to the compounds than observer No. 1. Thus the substances can also on the basis of these results be separated from other sweet-tasting compounds tested by group I (Table 22, col. 3*a*). This may indicate (p. 495) that these two groups of compounds affected different populations of receptors in a common area and/or different types of receptor sites which for genetic or other reasons existed in the two observers in different proportions.

2-Nitropropane evoked a bitter taste in 11 observers in all, a sweet-and-bitter taste in one observer out of 20 (Table 14) and a sweet taste in two observers out of 20. I therefore assign 2-nitropropane to the group of homologues discussed under this

section and not to the group of compounds treated in § 1. Phenylthiourea is also assigned to the group of compounds discussed in this section when presented to non-tasters. One of these non-tasters called the taste "sweet and bitter". According to Skude (1960), who performed similar experiments, four observers out of sixty-seven had the same opinion of the taste quality.

A line fairly parallel to that fitting the points for hypothetical compounds, the thresholds of which are equal to their solubilities in water, seems to be a rather good fit to the points in question in the diagram (Fig. 8). The substances seem to affect a part or parts of a similar nature in the excitable systems of at least two different populations of receptors, which on stimulation yield sweet and bitter sensations, respectively.

§ 3. The compounds concerned are phenol, pyrocatechol, hydroquinone, pyrogallol, phloroglucinol, *p*-nitrophenol, 2.5- and 2.6-dinitrophenol and sucrose. These are the remaining substances located between the compounds described under §§ 2 and 4 in the diagrams.

In Fig. 7 the points are rather scattered. The taste quality of the substances (Table 22, col. 2), like the results of the variance analyses (col. 3), is very divergent. To recapitulate briefly, it seems as if pyrocatechol has only an anaesthetizing effect and that the taste quality of 2.6-dinitrophenol cannot be stated. These compounds therefore receive no further mention in the discussion. Pyrogallol evokes a bitter sensation which is not observed on the same part of the tongue as that noted when other bitter substances are tested. Furthermore a sweet taste is observed when the substance is removed. The other compounds which have been included in Table 22 have the common property that they are sweet in the vicinity of the threshold value. However, in higher concentrations phenol is in addition anaesthetic, hydroquinone tastes salty (Group I) or bitter (Group II) and *p*-nitrophenol and 2.5-dinitrophenol yield a burning sensation.

The fact that a decrease of the threshold values for *p*-nitrophenol and hydroquinone was observed at the transition from the lower to the higher level of pH (cf. p. 502 and Table 22, col. 3*a*) deserves special attention. It may mean that the pK_a values of the components in the membrane which are involved in the relevant stimulation processes are different from those which are engaged in the stimulation by other substances. Furthermore the slopes of the *R-S* functions estimated for the compounds phenol, phloroglucinol, *o*- and *p*-nitrophenol and sucrose are approximately the same (cf. col. 5 and Fig. 5 (p. 513)). However, this cannot be taken as a proof that they all stimulate the same population of receptors. It only means that the integrated response of receptors put into action at different concentrations is of about the same magnitude.

As will be seen, the points of the substances considered are distributed in Fig. 8 in a similar way to that in Fig. 7. I therefore propose to draw (*a*) a line through the points representing the compounds phenol, phloroglucinol and sucrose, which all seem to be sweet also in higher concentrations (pyrogallol has not been included, owing to its peculiar taste) and (*b*) another line through the points for hydroquinone, *p*-nitrophenol and 2.5-dinitrophenol. The last-mentioned point is found near that of phloroglucinol in this diagram but not in that in Fig. 7. The taste evoked is also very similar to that of *p*-nitrophenol (Table 22, col. 2).

To sum up, it appears that the substances tested are arranged along six lines, of which two approximately coincide with each other. The substances located along such a line are looked upon as a group because they exhibit similar properties (Table

22). Group 1, described in § 1 (p. 517), shows an almost direct proportionality between the stimulating ability and the solubility in water of bitter substances.

Group 5 (§ 4) is fitted by two approximately coinciding lines, showing an inverse relationship with the proportionality constant near unity between the threshold value for sweet and bitter taste, respectively, and the number of moles per unit of volume in saturated solutions. Groups 2, 3 and 4 include sweet substances. Those in Group 4 ((*b*) in § 3) are arranged in a direction fairly parallel to the lines fitting sweet-and-bitter compounds. The lines representing groups 2 and 3 (§§ 2 and 3 respectively) have considerably lower slopes, showing that probably at least one other additional factor besides water solubility has to be considered in explaining the size of the regression coefficient.

In seeking to unravel the relationship between the stimulating ability and the physicochemical properties of the substances tested, a task of current importance seems to be to investigate how the "number of moles in saturated aqueous solutions of the compounds" finds expression as a functioning factor. One of several possible interpretations seems to be that compounds belonging to the different groups described (Fig. 7 and 8) have to pass into media of different compositions and thus also have to participate in different phase equilibria before reaching the actual sites of action. As a consequence, both the composition and the physicochemical properties of the medium surrounding a receptor site must play important rôles as regards the stimulating ability of the actual compounds.

Possibly the components of such a medium are polar in nature, at sites reacting with compounds in group No. 1 and less polar to non-polar at those reacting with compounds in groups Nos. 2–5. The fact that the compounds in group No. 1 have, on the average, a fairly higher stimulating potency than the rest, seems to support this view.

It is possible that the rate of reaction, between the stimulating molecules and an excitable system, which expresses the stimulating potency, is mostly determined by the ability of the compounds to reach the site of action. Of course, such an excitable system need not necessarily be located at the receptor membrane on the apical part of the taste cell. Diamant *et al.* (1963) and Hellekant (1965*b*) have, for example, presented arguments for the view that ethanol penetrates the epithelium of the tongue, and Hellekant (1965*b*) has considered the possibility that the compound stimulates an excitable system at the epithelial nerve-fibre endings. In electrophysiological experiments with dogs Diamant *et al.* (1963) has shown that ethanol produces electrical responses in fibres sensitive to quinine sulphate as well as to those sensitive to sucrose. From the standpoint of comparative physiology it is interesting to note that ethanol, according to the psychophysical experiments performed on man by Diamant *et al.* (1963) and by the present author, may evoke sweet, bitter and burning sensations simultaneously. The presence of the last-mentioned sensation means in fact that the stimulating molecules have to penetrate the epithelium to reach the trigeminal nociceptive fibres. Similar results to those obtained by Diamant *et al.* (1963) in experiments with dogs were obtained with cats by Hellekant (1965*b*), who also showed (1965*c*) that ethanol stimulates fibres in the trigeminal part of the lingual nerve which are sensitive to mechanical stimulation and to cooling. This compound thus seems to have a non-specific effect on the sensory organs. If the compounds which are discussed in this investigation under § 4 have to penetrate the non-polar parts of the receptor membrane to reach the site of action, the connection between stimulating ability and solubility found should in fact be expected. It would

be interesting to see whether results similar to those obtained in electrophysiological experiments with ethanol are obtained when other compounds belonging to this group are used as stimuli.

In interpreting the results, the specificity of the reaction between the stimulating molecules and the excitable system in the receptors has also to be considered. Proof of a pronounced specificity of reaction can be shown for some of the compounds studied. As an example, it may be mentioned that 1.2-dinitrobenzene is sweet, while the 1.3- and 1.4-dinitrobenzene derivatives are bitter. This is also the case in high concentrations. According to Trotter (1961), the atoms of nitrogen in 1.3- and 1.4-dinitrobenzene are situated in the plane of the benzene ring, while the plane through the atoms of the nitro group is twisted *c.* 11° out of the ring plane about the C–N bond. Since in the ortho derivatives the nitro groups are situated close to each other, and since furthermore each of them occupies a considerable space, we may assume that in this case the twisting with regard to the C–N bonds is much more pronounced. That this is necessarily the case can also be demonstrated by means of atomic models. It is difficult to say to what extent such minor differences in the stereostructure are responsible for the differences in the quality of taste of the isomers. The ortho and para compounds have furthermore a very similar distribution of π-electrons in the benzene nucleus. However, the ortho derivative has a strong dipole moment, while the para derivative, on account of the symmetrical distribution of the charged groups in the molecule, has no such moment. The three-dimensional distribution of the polar groups upon the aromatic nucleus can thus be considered as of great importance for the physicochemical properties of the compounds and for the way in which they act under different environmental conditions. Possibly the derivatives orientate themselves in different ways with regard to the polar or non-polar parts of a receptor site. The circumstance that no bitter component is found in the sweet taste evoked by saturated solutions of 1.2-dinitrobenzene, and no element of sweet taste in saturated solutions of the bitter-tasting 1.3- and 1.4-dinitrobenzenes shows that the specificity of the reaction between these molecules and the actual excitable system is high. The change in stereostructure at the transition from the ortho to the meta derivative is thus followed, disregarding the change of taste quality, by an abrupt change in the intensity of sensation. The corresponding change in configuration at the transition from the meta to the para compound, on the other hand, has no effect. In this case there seems to be an all-or-nothing response of the excitable system to a change in stereostructure.

Between the compounds in each group fitted by the lines in the diagrams in Figs. 7 and 8, large differences often seem to exist with regard to the stereoconfiguration and also the distribution of charges within the molecule. Whether these characteristics are responsible for the specificity directly or indirectly, alone or in co-operation, cannot be decided until a larger number of compounds have been included in each group in question. It is possible, for example, that the compounds hitherto found within the actual groups have affected different parts of an excitable system or different excitable systems situated in a similar molecular environment. If, on the other hand, it is not possible to divide a great number of compounds within each group into subgroups according to similarities in stereoconfiguration and/or distribution or charges within the molecule, the problem of specificity may be difficult to survey. We will then only have to consider the secondary effects of these characterisistics. Here the results (p. 428) obtained by Ehrensvärd (1942) will be mentioned. From experiments with oil chains of the type 0.0001-M KCl/salicyl aldehyde/conc.

KCl/salicyl aldehyde $+X$/0.0001-M KCl, (where X denotes any of the aliphatic primary n-alcohols C_1-C_{12}), he concludes that the configuration of the alcohol molecules is of importance for their lodging between the molecules of the salicyl aldehyde in the phase boundary between oil and water and indirectly for the assymmetric potential of the chain.

In Ehrensvärd's view, the factors which are responsible for the development of a phase-boundary potential when molecules are introduced into the liquid phase boundary, such as, for example, the "contribution of neutralization" in the above-mentioned cases, are also effective under similar conditions at the cell membrane of the chemoreceptors.

So far, only a comparatively few compounds have been investigated in the experiments discussed in the present investigation. The way in which the "number of moles in saturated aqueous solutions of the compounds" affects their stimulating ability must therefore be further studied. As has been earlier mentioned (p. 525), the effect of pH on the absolute threshold may be explained on the assumption that, before reaching the site of action, the stimulating molecules have to pass into a medium which is of different polarity at the two levels of pH examined. An attempt to adapt in detail the results of the analyses of variance to the above suggestion—that the compounds take part in certain phase equilibria before reaching the site of action—would, however, have to be preceded by very detailed examinations of the parts played by pH and temperature in the solubility of the compounds in water and in their distribution between phases of different polarities. Furthermore a detailed study of the chemical composition and submicroscopical and molecular structure of the taste cell membrane would have to be made. For the present, no such investigation seems to have been performed.

Finally, I examined how the lines of the diagrams in Figs. 7 and 8 fitted threshold values obtained by other authors for different compounds. This was done by expressing the thresholds in relative values and then anchoring them in the diagrams by means of a common substance, such as quinine hydrochloride or sucrose, for which the threshold values are given by many authors, besides those of other substances tested. However, such a treatment turned out to be meaningless, as values given by different authors for other common substances differ from each other to the extent of 10 or even 100 times. Obviously, either the differences between the subjects tested as regards their sensitivities to different substances were much greater than those found in the experiments with group I in the present investigation or the values found in the literature are not reliable.

Dependence of the taste quality on the concentration and on the removal of the stimulus

As can be seen from Table 5 (p. 450), sodium nitrite and potassium nitrite can give rise to sweet taste in fairly low concentrations. In the preliminary experiments (p. 507) aimed at the study of the connection between the intensity of the sensation and the strength of the stimulus, it was, however, observed that the sweet taste of sodium nitrite disappeared when the concentration was increased, while at the same time the sensation described as metallic became increasingly obvious. In the course of the tasting of a great number of electrolytes, among them the majority of the halides of the alkali metals, Renqvist (1919) found the taste to be sweet within the range of concentration from the threshold value up to 0.02–0.04 M. Within

the range of concentration covering approximately 0.03–0.2 M, there appeared a new quality of taste, which was assumed to depend upon the quotient between the speed of migration of the two kinds of ions. If the speed of migration of the anion was equal to, greater or much greater than that of the cation, the sweet taste became bitter or saline or remained sweet, respectively. As examples of substances of the last type Renqvist mentions NaOH and KOH, which proved to be sweet up to the concentrations (about 0.05 M) above which they produced an "etching" sensation.

Since the majority of the electrolytes studied by Renqvist are considered to be sweet in the neighbourhood of the absolute threshold, the effect of sodium nitrite and potassium nitrite may possibly be interpreted as an unspecified ion effect leading to a change of the potential in the phase boundary between the taste cell and the external medium. It is tempting to see similarities between the phenomenon of "quality of taste depending upon concentration" (cf. p. 507) and the type of connection between change of potential and change of the concentration of KCl obtained by Ehrensvärd in oil-chain systems of the type 1-M KCl/oil/x-M KCl, in which the oil was represented by benzyl alcohol (cf. Ehrensvärd 1942, p. 69, Fig. 7*c*). Within the range of concentration 10^{-5} – 1-M KCl upon the dilute side of the chain, the change of potential passed through a maximum. If this should be the case also in the interior of the taste cell/cell membrane/external medium system, and if the change of potential in the neighbourhood of this maximum should by itself suffice for stimulation, the phenomenon of "quality of taste depending upon concentration" would become manifest. It is, however, also conceivable that the sweet taste of higher concentrations of the compound is suppressed in the central nervous system by the simultaneous increase of the impulses from the receptors which on activation produce the metallic sensation. A check on these hypotheses may be possible by studying, by electrophysiological methods, the connection between the frequency of discharge and the strength of stimulus for the above-mentioned electrolytes in single-fibre preparations or in receptor cells which have proved to react to sweet-tasting substances only. If in this case the preparations are not specifically sensitive to the electrolytes in question within a certain range of concentration in the neighbourhood of the absolute threshold, this would be in favour of the supposition that in higher concentrations the sweet taste disappears by central inhibition of the activity in "sweet" fibres.

Pyrogallol, in which the substituents are placed upon three neighbouring carbon atoms, has a bitter taste and produces a sweet taste on its removal from the epithelium (Table 14 (p. 478) and p. 487). In experiments not reported here I observed that benzyl alcohol also causes a sweet taste, when removed by rinsing the oral cavity. Certain buffers, for example, mixtures of primary and secondary phosphate, also show similar properties.

Renqvist (1919) also observed that, on being rinsed out of the oral cavity, several electrolytes cause a sweet taste. This happens when medium concentrations are used for the tasting. In lower concentrations these substances may also, when applied to the sensory surfaces, cause a sweet taste. The sweet after-taste should thus be caused by the fact that at the dilution the concentration of the solution at the surface of the receptor passes through the range of concentration for sweet taste. Renqvist also expresses the opinion that non-electrolytes are unable to produce a sweet after-taste "da sie im Geschmackssystem kein elektrisches Potential bilden können". My results obviously contradict this idea. A possible explanation may be that the substances hyper-polarize the receptor membrane. The removal of the substances

would then be accompanied by a depolarization, which, especially if the membrane had time to adapt itself to the new conditions, would be sufficiently great to initiate action potentials.

SUMMARY

Chapter I

The possible factors involved in the discrimination of taste quality and taste intensity are discussed. The general ideas about the connection between the chemical constitutions and the stimulating abilities of sweet and bitter compounds are reviewed.

Chapter II

1. A standardized method for the estimation of thresholds of taste in man is described. By this method, the absolute thresholds of the two observers forming group I were estimated for some sweet, sweet and bitter, and bitter compounds under each of the experimental conditions (1) "distilled" water (kind of water used as solvent and for rinsing) at 20.4°C and pH 5.1 (before the tasting), (2) "distilled" water at 36.9°C and pH 5.1, (3) tap-water at 20.4°C and (4) tap-water at 36.9°C. The compounds tested in this connection were a number of benzene derivatives with nitro and/or hydroxy substituents, then sucrose, quinine hydrochloride, and phenylthiourea.

2. The thresholds of the 20 observers forming group II were estimated under the experimental conditions "distilled" water at 20.8°C and pHs 5.1 and 7.8 for most of the compounds mentioned above and under the condition "distilled" water at 20.8°C and pH 5.1 for the smaller alkyles ($C_1 - C_3$) containing one of the substituents at a time. $NaNO_2$, KNO_2 and NaCl were also tested in this connection.

3. The stability of the compounds in aqueous solutions with different pH values was checked. In dilute solutions in contact with air a few of the hydroxybenzenes tested are unstable in the vicinity of the pH ranges examined.

Chapter III

The way in which the observers' assessments were made use of when defining the threshold for a compound is described. This definition is put into practice in calculating the mean threshold values of individual observers in group I and of group II as a whole.

Chapter IV

1. The concentration of the substance remaining in the oral cavity as a function of the number of rinsings performed after a solution of a compound had been tasted by the above procedure was studied. It is shown that about 1‰ of the concentration of an assessed solution added itself to the concentration in the preparation assessed after it.

An account is also given of the temperatures, the pH values, the concentrations of Na^+, K^+, Ca^{++} and Cl^- and the ratio $C_B - /C_{BH}$ in the tasted solutions. The concentrations of the compounds in these solutions represent the mean threshold values of group II as a whole and of the two observers of group I under the actual experimental conditions.

Chapter V

1. The experimental temperature, 35°C, of the epithelial surfaces in the mouth is found to be more favourable than 27°C for obtaining a low standard deviation of the absolute threshold of the observers in group I, when expressed as a fraction of the sample mean (coefficient of variation). The result is in accordance with the findings that the temperature of the epithelial surfaces in the mouth are rather more constant during experiments when solutions made up with water at 36.9°C are tasted than when water at 20.4°C is used as solvent and for rinsing.

2. There was a trend towards a lowering of the level of the thresholds of the observers in group I during the experiments in which tap-water was used as solvent and for rinsing. The actual trend did not, however, appear in experiments in which distilled water was used as solvent and for rinsing. Possible explanations of these results are presented.

3. No noticeable change in value of the thresholds of observers in group I for the compounds tested was found during the rather long experiments, although some of these compounds are oxidized comparatively rapidly.

4. The thresholds obtained of observers in group II for different substances did not change significantly between the first and second determination, probably on account of the short span of time between the two determinations.

5. The mean threshold value of group II for resorcinol at pH 5.1 did not noticeably change in any direction in the course of the rather long period of testing (March–May), according to repeated checks. Thus there was no noticeable effect of training upon the threshold.

6. The coefficients of variation (C.V.) for group II as a whole fluctuate considerably around the mean value of 59 %, which represents all of the compounds tested at pH 5.1, except phenylthiourea. The group shows an exceptionally high C.V. (264 %) for this compound, because of the fact that two of the observers turned out to be non-tasters.

Chapter VI

Most of the compounds evoke a sweet, a sweet and bitter or a bitter taste. In addition, many of them affect other sense modalities. The sweet taste is localized to the tip of the tongue, while the bitter taste is found at its base. Pyrogallol, although bitter, forms an exception, in that it also stimulates the distal edges of the tongue. The stimulated receptors seem therefore to have another distribution on the tongue than those excited by the other bitter compounds. Pyrogallol furthermore causes a sweet taste when removed from the receptor surface.

Chapter VII

The effect of pH on the threshold values of observers in group II was studied with the aid of Student's *t*-test for some of the compounds tested.

The effect of the factors of "temperature", "quality of the water", when used as solvent and for rinsing, and "observer" on the values of the absolute thresholds of observers in group I was also studied with the aid of analysis of variance.

The observation that one of the observers in group I showed a higher acuity than the other only for some of the sweet compounds indicates that these do not affect the same populations of receptors and/or the same types of receptor sites as the other sweet compounds tested.

The dependence of the absolute thresholds upon the temperature is, on the whole, not very pronounced, although the temperature of the epithelial surface of the tongue is at least 8°C higher when the temperature of the introduced liquids is *c.* 36.9°C than when it is *c.* 20.4°C. Possible explanations of this phenomenon are presented. In each case, when a significant effect is obtained, the threshold value is found to be lower at the higher level of temperature studied.

In the majority of the factorials the effect of the quality of the water made its appearance, either directly or hidden in the interaction between water and temperature (W × T). In most of these cases the mean threshold values were higher when tap-water was used as solvent and for rinsing than when distilled water was used. Exceptions were the compounds hydroquinone and *p*-nitrophenol. Similar results were obtained in studying the effects of pH on the threshold values of observers in group II, according to the *t*-tests applied. Possible explanations for the appearance of the effect of the water's quality, probably representing an effect of pH, are given.

Chapter VIII

1. The connection between the intensity of sensation and the strength of the stimulus was estimated by psychophysical methods for some of the compounds tested. The possibility of the existence of a simple connection between the intensity of sensation and the frequency of the discharge in the taste nerves is discussed. The fit of the data obtained for the compounds to the absorption theorems of Beidler and Freundlich was also studied.

The slopes of the curves representing the connection between the logarithms of the intensity of sensation and the logarithms of the strength of the stimulus for some of the sweet compounds studied agree between themselves but deviate from the slopes of the other compounds in question. These two groups of compounds thus do not affect the excitable system in the receptors in similar ways. Similar conclusions are drawn as regards the bitter compounds tested.

Chapter IX

The solubilities of the compounds in water were determined. No connection between the stimulating ability and the solubility, represented by the number of moles present per unit of volume in saturated solutions, is found when *all* sweet or bitter compounds are considered. However, a linear relation seems to exist between the logarithms of the mean threshold values and the logarithms of the corresponding values for the solubilities in each of the five groups of compounds obtained, when consideration is given to (1) their taste qualities, (2) the stimulated area of the tongue, (3) the results of the variance analyses of the influence of the observer, the quality of the water and the temperature on the threshold values, and (4) the relationship between the intensity of sensation and the strength of the stimulus. A possible explanation of these results is presented.

The reaction between the stimulating molecules and the excitable system in the receptors seems to be highly specific. Similarities between the compounds in each of the five groups, as regarded stereoconfiguration and distribution of charges, was, however, not found. Thus there is a possibility that the compounds hitherto found in the actual groups affected different parts of an excitable system or different excitable systems situated in similar molecular environments.

Finally the modes of action of sodium nitrite and pyrogallol on the taste apparatus are discussed.

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